CHAPTER II

EXPERIMENT

1.Materials

1.1 Model Drug

- Terbutaline sulfate

(Supplied by Utopian, Thailand, Batch No. TSI 0397004)

1.2 Additives

- Chitosan SEACURE 143

(Pronova biopolymer, Drammen, Norway, Batch No. 731727G)

- Chitosan SEACURE 243

(Pronova biopolymer, Drammen, Norway, Batch No. 731127G)

- Chitosan SEACURE 343

(Pronova biopolymer, Drammen, Norway, Batch No. 731517G)

- Polyvinyl alcohol average molecular weight 70,000-100,000

(Sigma chemical Co., St. Louis, USA)

- Polyvinylpyrrolidone K-90 (PVP K-90)

(Supplied by Utopian, Thailand, Lot. No. C50331)

- Glycerin

(Srichand United Dispensary Co., Ltd., Thailand, Lot. No. GB33)

- Propylene glycol

(Srichand United Dispensary Co., Ltd., Thailand, Lot. No. PL33)

- Lactic acid

(W. Germany)

- Baysilone-paste ®

(Bayer, Germany, Lot. No. 7663440)

1.3 UV-visible Spectroscopy Analysis

- 4-Aminoantipyrine

(Fluka, Switzerland, Lot. No. 2014523)

- Hydrochloric acid

(BDH Laboratory supplies, England, Lot. No. K23000252)

- Potassium ferricyanide

(Fluka, Switzerland, Lot. No. 2373233)

- Tris (hydroxymethyl) aminomethane

(Fluka, Switzerland, Lot. No. 2010644)

1.4 High Performance Liquid Chromatography Analysis

- Methanol HPLC grade

(BDH Laboratory supplies, England, Lot. No. L647202)

- Ortho-phosphoric acid 85%

(E.Merck, Germany, Lot. No. 709K4247473)

- Potassium phosphate monobasic

(Merck, Germany, Lot. No. A894271605)

- Salicylic acid

(Fluka AG., Switzerland, Lot. No. 236018583)

- Sodium chloride

(E.Merck, Germany, Lot. No. K20420804347)

2. Apparatus

- Analytical balance (Sartorius A 200 S, Sartorius Ltd., Co., Germany)
- Diffusion cell (Modified from Keshary-Chien diffusion cell)
- Fourier transform infrared spectrometer (Model 1760X, Perkin Elmer, USA)
- HPLC (Millipore Waters Chromatograph Division, Milford, Massachusetts, USA) composed of:
    - Model 600 E multisolvent delivery system
    - Water 484 tunable absorbance detector
    - Model 712 Water intelligent sample processor (WISP^{TM})
    - Water 746 data module
- Incubator (Memmert model BM 600, Germany)
- Magnetic stirrer (Heidolph model MR 3001, Germany)
- Micrometer (Teclock Corp., Japan)
- pH meter (Pye model 132, Pye Unichem Limited, England)
- Powder X-ray diffractometer (Philips, Netherlands) composed of:
    - Generator model PW 1830
    - X-ray Cu-tube model PW 2233
    - Vertical goniometer model PW 1050/70
    - Proportional detector model PW 1965/60
    - Compact chart recorder model PM 8202
- Scanning electron microscope (Model JSM-6400, Jeol, Japan)
- Sonicator (Transsonic Digital model T680/H, Elma, Germany)
- Tensometer (Instron model 5565, Instron Corp., Canton, MA, USA)
- Thermal analyzer (DSC model 92, Setaram, France)
- Ultraviolet-visible spectrophotometer (Shimadzu UV-160 A, Shimadzu Corp., Japan)

3. Methods

For the design of a self-adhesive transdermal drug loading film, this system composed of backing material, drug-loaded adhesive matrix and release liner.

3.1 Preparation of Backing Material

The glass petri dish with diameter 89 mm was greased with baysilone-paste® and then covered with backing material. Blotting paper was used as a backing material in this experiment. The average thickness of blotting paper was 50 μm.

3.2 Formulation of Drug-free Transdermal Patches

3.2.1 Preliminary Investigation for Suitable Ratio of Chitosan and Polyvinyl Derivatives

The formulations of drug-free transdermal patch are presented in Tables 2 and 3. Each formulation consisted of chitosan and polyvinyl derivatives (polyvinyl alcohol or PVP K-90). The amount of total polymer was fixed at 5 %w/w. The ratios of chitosan and polymer were varied from 1:9 to 9:1. Chitosan (SEACURE 143) was used in preliminary investigation. In some formulations, plasticizers were used to increase adhesiveness. Propylene glycol was used in a concentration of 5 %w/w and glycerin was used in a concentrations of 5, 10, 15 and 20 %w/w, respectively. The thickness of the drug-free transdermal patch was controlled to be about 200 μm ± 10 % (250 μm ± 10 % including backing material).

Table 2 Formulations of drug-free chitosan and polyvinyl alcohol casting solution using different ratios of chitosan and polyvinyl alcohol

Ingredients	Formulation CA																	
(g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVA	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Propylene glycol	-	-	-	-	-	-	-	-	-	5	5	5	5	5	5	5	5	5
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Ingredients	Formulation CA																	
(g)	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVA	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Glycerin	5	5	5	5	5	5	5	5	5	10	10	10	10	10	10	10	10	10
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: CA = chitosan and polyvinyl alcohol

Table 2 (cont.) Formulations of drug-free chitosan and polyvinyl alcohol casting solution using different ratios of chitosan and polyvinyl alcohol

Ingredients	Formulation CA																	
(g)	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVA	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Glycerin	15	15	15	15	15	15	15	15	15	20	20	20	20	20	20	20	20	20
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: CA = chitosan and polyvinyl alcohol

Table 3 Formulations of drug-free chitosan and PVP K-90 casting solution using different ratios of chitosan and PVP K-90

Ingredients	Formulation CP																	
(g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVP K-90	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Propylene glycol	-	-	-	-	-	-	-	-	-	5	5	5	5	5	5	5	5	5
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Ingredients	Formulation CP																	
(g)	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVP K-90	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Glycerin	5	5	5	5	5	5	5	5	5	10	10	10	10	10	10	10	10	10
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: CP = chitosan and PVP K-90

Table 3 (cont.) **Formulations of drug-free chitosan and PVP K-90 casting solution using different ratios of chitosan and PVP K-90**

Ingredients	Formulation CP																	
(g)	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
SEACURE 143	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
PVP K-90	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Glycerin	15	15	15	15	15	15	15	15	15	20	20	20	20	20	20	20	20	20
Lactic acid	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: CP = chitosan and PVP K-90

3.2.2 Preliminary Investigation for Suitable Ratio of Chitosan and Polyvinyl Derivatives by Fixing the Amount of Chitosan and Varying Amounts of Polyvinyl Derivatives

The formulations are shown in Tables 4 and 5. The amount of chitosan was fixed at 0.5 or 1.0 %w/w and the amounts of polyvinyl derivatives were varied from 9 to 20 %w/w. Glycerin in a concentration of 10 %w/w was used as plasticizer.

3.2.3 Preliminary Preparation of Drug-free Transdermal Patch by Using Three Grades of Chitosan and Fixing the Amount of PVP K-90

The formulations are shown in Table 6. To study the effect of chitosan molecular weight, three grades of chitosan (SEACURE 143, SEACURE 243 and SEACURE 343), were used to prepare transdermal patch. All grades of chitosan were used in a concentrations of 0.1, 0.3, 0.5 and 0.7 %w/w respectively. The amount of PVP K-90 was fixed at 10 or 15 %w/w.

3.3 Formulation of Terbutaline Sulfate Transdermal Patch

A transdermal patch with a contact surface area of 1.77 cm^2 and a total terbutaline sulfate content of 15 mg was prepared in this study. The formulations are presented in Table 7. PVP K-90 was selected to prepare drug-loaded transdermal patch. To study the effect of molecular weight of chitosan, three grades of chitosan were used. All grades of chitosan were used in a concentrations of 0.1, 0.3, 0.5 and 0.7 %w/w respectively. The amount of PVP K-90 was fixed at 10 or 15 %w/w. Glycerin was used in a concentrations of 5 and 10 %w/w.

Table 4 Formulations of drug-free chitosan and polyvinyl alcohol casting solution using different amounts of polyvinyl alcohol

Ingredients	Formulation FCA																							
(g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
SEACURE 143	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PVA	9	10	11	12	13	14	15	16	17	18	19	20	9	10	11	12	13	14	15	16	17	18	19	20
Glycerin	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Lactic acid	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: FCA = fix chitosan and polyvinyl alcohol

Table 5 Formulations of drug-free chitosan and PVP K-90 casting solution using different amounts of PVP K-90

Ingredients	Formulation FCP																							
(g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
SEACURE 143	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PVP K-90	9	10	11	12	13	14	15	16	17	18	19	20	9	10	11	12	13	14	15	16	17	18	19	20
Glycerin	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Lactic acid	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: FCP = fix chitosan and PVP K-90

Table 6 Formulations of drug-free chitosan and PVP K-90 casting solution using different molecular weights and amounts of chitosan

Ingredients	Formulation VCP																							
(g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
SEACURE 143	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-
SEACURE 243	-	-	-	-	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7	-	-	-	-
SEACURE 343	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7
PVP K-90	10	10	10	10	10	10	10	10	10	10	10	10	15	15	15	15	15	15	15	15	15	15	15	15
Glycerin	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Lactic acid	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Remark: VCP = vary chitosan and PVP K-90

Table 7 Formulations of drug-loaded chitosan and PVP K-90 casting mixture

Ingredients	Formulation											
(g)	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	B_1	B_2	B_3	B_4
Terbutaline sulfate	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56
SEACURE 143	0.1	0.3	0.5	0.7	0.1	0.3	0.5	0.7	-	-	-	-
SEACURE 243	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7
PVP K-90	10	10	10	10	10	10	10	10	10	10	10	10
Glycerin	10	10	10	10	5	5	5	5	10	10	10	10
Lactic acid	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100

Ingredients	Formulation											
(g)	B_5	B_6	B_7	B_8	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
Terbutaline sulfate	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56
SEACURE 243	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-
SEACURE 343	-	-	-	-	0.1	0.3	0.5	0.7	0.1	0.3	0.5	0.7
PVP K-90	10	10	10	10	10	10	10	10	10	10	10	10
Glycerin	5	5	5	5	10	10	10	10	5	5	5	5
Lactic acid	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100

Table 7 (cont.) Formulations of drug-loaded chitosan and PVP K-90 casting mixture

Ingredients	Formulation											
(g)	AA_1	AA_2	AA_3	AA_4	BB_1	BB_2	BB_3	BB_4	CC_1	CC_2	CC_3	CC_4
Terbutaline sulfate	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56	10.56
SEACURE 143	0.1	0.3	0.5	0.7	-	-	-	-	-	-	-	-
SEACURE 243	-	-	-	-	0.1	0.3	0.5	0.7	-	-	-	-
SEACURE 343	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.7
PVP K-90	15	15	15	15	15	15	15	15	15	15	15	15
Glycerin	10	10	10	10	10	10	10	10	10	10	10	10
Lactic acid	.05	.15	.25	.35	.05	.15	.25	.35	.05	.15	.25	.35
Deionized water q.s. to	100	100	100	100	100	100	100	100	100	100	100	100

Remark: A and AA = SEACURE 143; B and BB = SEACURE 243; C and CC = SEACURE 343

A, B and C = 10 %w/w PVP K-90; AA, BB and CC = 15 %w/w PVP K-90

$_{1, 2, 3}$ and $_4$ = 0.1, 0.3, 0.5 and 0.7 %w/w chitosan, respectively

3.4 Method for Preparing Transdermal Patches

3.4.1 Drug-free Chitosan and Polyvinyl Alcohol Transdermal Patch

Chitosan was dispersed in a lactic acid solution with constant stirring using magnetic stirrer for 24 hours. The resultant viscous solution was filtered through gauze cloth. The filtrate was left to stand until all air bubbles had disappeared. A required amount of polyvinyl alcohol was wetted with deionized water and dissolved by heating at 70 °C and allowed to cool. An aqueous solution of polyvinyl alcohol was mixed with chitosan solution. In some formulations, propylene glycol or glycerin was used as plasticizer. The polymer blend solution was stirred at room temperature, followed by the addition of one of plasticizers to form a uniform solution. The casting solution was left to stand until air bubbles had disappeared. The casting solution of appropriate weight was poured onto the glass petri dish which was backed with backing material, then stored in an oven at 40 °C for 12 hours on the levelled surface to dry. The dried film was peeled off from the glass surface, packed in a release liner covering and stored in a desiccator. Physical appearances and adhesiveness of film were used to select the film for further evaluation.

3.4.2 Drug-free Chitosan and PVP K-90 Transdermal Patch

An aqueous solution of chitosan was prepared as above. A required amount of PVP K-90 was dissolved in deionized water. An aqueous solution of PVP K-90 was left to stand until all air bubbles had disappeared. Aqueous solution of PVP K-90 was mixed with chitosan solution. In some formulations, propylene glycol or glycerin was used as plasticizer. The polymer blend solution was stirred at room temperature, followed by the addition of one of plasticizers to form a uniform

solution. The casting solution was left to stand until all air bubbles had disappeared. The later processes were consecutively performed as in 3.4.1.

3.4.3 Drug-loaded Chitosan and PVP K-90 Transdermal Patch

An aqueous solution of chitosan was prepared as above. A required amount of PVP K-90 was dissolved in deionized water. An aqueous solution of PVP K-90 was mixed with chitosan aqueous solution. The polymer blend solution was stirred at room temperature, followed by the addition of glycerin to form a uniform solution. Later a known amount of terbutaline sulfate was slowly added while stirring until the preparation was homogeneous. Then the casting mixture was left to stand until all air bubbles had disappeared. The casting mixture of appropriate weight was poured onto the glass petri dish in which backing material was spread to cover the glass area. Then the film was allowed to dry in an oven at 40 $^{\circ}$C for 12 hours on the levelled surface. The terbutaline sulfate transdermal patch was peeled off from the glass surface, packed in a release liner covering and stored in a desiccator at room temperature for further evaluation.

4. Evaluation of Physicochemical Properties of Terbutaline Sulfate Transdermal Patches

4.1 Determination of Thickness

The thickness of transdermal patches was measured by using a micrometer. Each sample was determined at five separate points, one point at centre and the other points around the central point to ensure the thickness of 250 μm $\pm$ 10 % (include backing material). Triplicates were performed.

4.2 Determination of Moisture Absorption/Loss (Kanig and Goodman, 1962)

Terbutaline sulfate transdermal patches were cut into 1x3 cm strips. The strips were then conditioned by placing them in an oven at 40 °C for 12 hours, which were the temperature and the drying time originally used in drying the wet films. This step was carried out to ensure uniformity of the drying of the films within each group before testing. The conditioned sample strips were then suspended by means of fine wire in relative humidity chambers. Four chambers were set up to produce relative humidities of 0, 20, 52 and 93 % respectively. The relative humidity in the chamber was controlled by the use of silica gel and saturated solutions of potassium acetate, magnesium nitrate and potassium nitrate respectively. The conditioned samples were accurately weighed, placed in the relative humidity chambers, and then removed and weighed again at the end of one and four week intervals. Increase and decrease in weight, changes in physical appearance were then observed. Percent moisture absorption/loss was calculated by means of the following formula:

$$\frac{(\text{weight of exposed film - weight of conditioned film}) \times 100}{\text{weight of conditioned film}}$$

This experiment was performed in triplicate.

4.3 Determination of Mechanical Properties by Measuring Ultimate Tensile Strength and Percent Elongation at Break

Ultimate tensile strength and percent elongation at break were measured on an Instron tensile tester. The tester was equipped with a 1 kg tension load cell. The cross-head speed was controlled at 20 mm/min. Film specimens were cut into 2x20

mm rectangular shape. The thickness of each specimen was the average value of four separate measurements using a micrometer. Then the test specimen was clamped by the upper and lower grip. The length of film between the grips was 10 mm. Six strips for each terbutaline sulfate transdermal patch were examined for their ultimate tensile strength and percent elongation at break of the films and the mean was automatically computed by the Instron series IX material testing software.

4.4 Determination of Peel Adhesion Property

The film was applied to stainless steel plate and then pulled from the substrate at a 180° angle using an Instron tester to measure the force. A 1 kg tension load cell was chosen and the cross-head speed was set at 20 mm/min. Six strips were tested and the mean was obtained.

4.5 Surface Morphology

The surface morphology and cross section of selected terbutaline sulfate transdermal patches were studied by using a scanning electron microscope (SEM). Transdermal patches were mounted on a metal stubs, coated with gold and observed under SEM.

4.6 Infrared Spectrometry

Infrared spectrometry was used to study the interaction between the components in the formulation. The Fourier transform infrared (FT-IR) spectrometer was employed to observe infrared spectra. In this study, the disc method by using potassium bromide as a diluent was used for pure substances, polymers blend, drug-

polymers blends and selected terbutaline sulfate transdermal patches. SEACURE 243 was used to study the polymer-polymer and drug-polymer interaction. The halide disc containing the sample was placed in FT-IR case and scanned from 4000 to 400 cm^{-1}. IR spectrum of glycerin (plasticizer) was not examined because it was in the liquid form that could not be evaluated by this method.

4.7 Powder X-ray Diffraction Analysis

The crystallinity of three grades of chitosan was evaluated by a powder X-ray diffractometer. The X-ray diffractograms of chitosan powders and films were taken at room temperature using a CuKα radiation generated at 30 kV and 30 mA. The samples were scanned from 4° to 46° (2θ) at a speed of 2° (2θ)/min.

4.8 Differential Scanning Calorimetry

Differential scanning calorimetry thermograms were observed by using the differential scanning calorimeter. Each sample was investigated for its melting point. Samples, including pure substances, polymers blend, drug-polymers blends and selected terbutaline sulfate transdermal patches, were accurately weighed and put into the equipment using a given condition:

Heating rate	:	10°C/min
Temperature	:	30 - 300°C
Atmosphere	:	N_2 15 ml/min
Sample cell	:	Aluminium closed pan

4.9 Determination of Terbutaline Sulfate Content in Transdermal Patches

The UV spectrophotometric method for determining terbutaline sulfate content in transdermal patches used in this study was modified from USP XXII.

Buffer pH 9.5 was prepared by dissolving 36.3 g of tris (hydroxymethyl) aminomethane with deionized water in a 1,000 ml volumetric flask and then the buffer solution was adjusted up to volume. The pH was adjusted to 9.5 ± 0.1 with 1 N hydrochloric acid.

The 4-aminoantipyrine solution was freshly prepared in deionized water having a concentration of 20 mg/ml.

The potassium ferricyanide solution was freshly prepared in deionized water having a concentration of 80 mg/ml.

Calibration Curve

Terbutaline sulfate was accurately weighed and dissolved in 0.01 N hydrochloric acid to get final concentration of 100 μg/ml. The standard solution containing 100-500 μg of terbutaline sulfate were transferred into a series of 25 ml volumetric flask. The final concentration of terbutaline sulfate of each flask was 4, 6, 8, 10, 12, 16 and 20 μg/ml. To each flask appropriate volume of pH 9.5 buffer was added to bring the total volume to 5.0 ml and then 17.5 ml of pH 9.5 buffer and 0.5 ml of 4-aminoantipyrine solution were added and mixed. The flask was vigorously swirled and 0.5 ml of potassium ferricyanide solution was added. Finally, the solution was adjusted up with pH 9.5 buffer to volume and mixed. Seventy-five seconds,

accurately timed, following the addition of the potassium ferricyanide solution, the absorbance of the solution was measured at the wavelength of 550 nm against the reagent blank with UV spectrophotometer. The reagent blank was prepared as the aforementioned procedure and 5 ml of 0.01 N hydrochloric acid was used instead of the standard solution. Each concentration was determined in triplicate.

The calibration curve of terbutaline sulfate was constructed by plotting the concentration of terbutaline sulfate versus the absorbance of terbutaline sulfate. A linear regression equation was used to calculate the concentration of terbutaline sulfate in each sample. The concentration versus absorbance of terbutaline sulfate in 0.01 N hydrochloric acid at 550 nm is presented in Table 24 (Appendix A). The calibration curve of terbutaline sulfate after regression analysis is illustrated in Figure 70 (Appendix A).

Assay of Terbutaline Sulfate in Transdermal Patches

The terbutaline sulfate transdermal patch was cut into a diameter of 1.5 cm circular piece which contained 15 mg of terbutaline sulfate. Then sample specimen was placed into a flask and 100 ml of 0.01 N hydrochloric acid was added. The sample was dissolved by sonication for 30 minutes and filtered.

Each sample was diluted to suitable concentration which gave the absorbance between 0.2-0.8. The absorbance of sample solution was measured by the procedure described in calibration curve and sample solution was used instead of standard solution. The concentration of terbutaline sulfate was determined by using a linear regression equation.

5. *In-vitro* Evaluation

5.1 Design of Diffusion Cell

An *in-vitro* skin permeation study was carried out using a diffusion cell modified from Keshary-Chien diffusion cell. This diffusion cell consisted of two compartments, the donor compartment in the upper and the receptor compartment in the lower. The capacity of the receptor compartment was 15 ml. The diameter and cross sectional area of the receptor compartment were 1.5 cm and 1.77 cm^2 respectively.

5.2 Preparation of Shed Snake Skin

Shed snake skin of *Elaphe obsoleta* was used as a model membrane for *in-vitro* skin permeation study. Shed snake skin was stored at -20 °C prior to use and the dorsal portion was cut into 3.5 cm diameter circular piece, hydrated in 100 ml receptor solution at room temperature about fourteen hours.

5.3 Preparation of Receptor Solution

Normal saline was used as the receptor solution in the receptor compartment of the diffusion cell.

5.4 The Permeability of Terbutaline Sulfate through Shed Snake Skin

The permeability of terbutaline sulfate through shed snake skin was measured *in-vitro* to examine the permeation profile of terbutaline sulfate. The saturated

terbutaline sulfate solution was prepared by dissolving 8 g of drug in 20 ml of reversed osmosis treated water. This solution was shaken at ambient temperature about 48 hours. Shed snake skin was mounted on the receptor compartment of the diffusion cell, with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor compartment was positioned and clamped. Normal saline solution was introduced into the receptor compartment which was thermostated at 37 ± 1 °C by a circulating waterbath. The appropriate amount of saturated terbutaline sulfate solution was pipetted into the donor compartment. The magnetic bar at the bottom of receptor compartment was controlled to rotate at 750 rpm.

The exact volume of samples was withdrawn at 1, 2, 4, 6, 8, 12, 16, 20 and 24 hours. Then the equal volume of fresh normal saline solution was replaced immediately. The cumulative amount of permeated terbutaline sulfate was determined by HPLC method. The permeability study was conducted in triplicate.

5.5 The *In-vitro* Permeation Study of Terbutaline Sulfate Transdermal Patches

Shed snake skin was mounted on the receptor compartment of the diffusion cell, with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The receptor solution was introduced into the receptor compartment which was thermostated at 37 ± 1 °C by a circulating waterbath. Terbutaline sulfate transdermal film was cut into a diameter of 1.5 cm circular piece and placed with the terbutaline-releasing surface in intimate contact with the skin and the donor cap was positioned and clamped.

At a predetermined time interval 1, 2, 4, 6, 8, 12, 16, 20 and 24 hours, 2 ml of

sample was withdrawn from the receptor solution and replaced immediately with a same volume of the fresh normal saline solution to keep the volume in the receptor compartment constant and to ensure a good contact between the dermal side of the skin and the receptor solution.

The terbutaline sulfate concentration in the sample was determined by HPLC method. This experiment was performed in triplicates.

5.6 HPLC Analysis of Terbutaline Sulfate

Chromatographic Conditions

The chromatographic system in this study consisted of

Chromatographic column	: Lichrospher® 100RP-18, particle size 5 μm with dimension of 150x4.6 mm, Merck
Internal standard	: Salicylic acid
Flow rate	: 2.0 ml/min
Injection volume	: 200 μl
Pressure	: 2,200 psi
Detector	: UV detector wavelength was set at 278 nm
Chart speed	: 0.25 cm/min

The mobile phase was 8 %v/v methanol in 0.02 M potassium phosphate monobasic in reversed osmosis treated water. The pH was adjusted to 3.6 with a 1.77 % aqueous solution of phosphoric acid. The mobile phase was freshly prepared, filtered through a 0.45 μm membrane filter and then the mobile phase was degassed by sonication for 15 minutes prior to use.

Calibration Curve

The standard solutions of terbutaline sulfate were freshly prepared on each day of sample analysis by diluting the stock solution with mobile phase to the desired concentrations of 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 μg/ml. Salicylic acid in methanol in a final concentration of 0.50 μg/ml was added to each standard solution. A 200 μl of standard solution was injected into HPLC column.

The calibration curve of terbutaline sulfate was constructed by plotting the concentration of terbutaline sulfate versus the peak area ratio of terbutaline sulfate and salicylic acid. The concentration versus the peak area ratio of terbutaline sulfate and salicylic acid at 278 nm is presented in Table 25 (Appendix A). A linear regression equation was used to calculate the concentration of terbutaline sulfate in each elution sample. The calibration curve and representative chromatograms are shown in Figures 71 and 72 (Appendix A) respectively.

Sample Preparation

The sample was accurately pipetted into a 5 ml volumetric flask and then salicylic acid in methanol was added to the final concentration of 0.50 μg/ml. The mixture solution was diluted with mobile phase to the volume of volumetric flask. Each elution sample had to be appropriate dilution for analysis the concentration of terbutaline sulfate.

The peak area ratio of terbutaline sulfate and salicylic acid was used to examined the concentration of terbutaline sulfate in the sample through the linear regression equation obtained from the calibration curve in that same day. Then the

cumulative amount of terbutaline sulfate permeating through shed snake skin was calculated from terbutaline sulfate concentration in each sampling.