CHAPTER III

EXPERIMENTAL

Materials

1. Lidocaine base : batch No. 101K0120. Sigma, Germany.
2. Lidocaine hydrochloride : Batch No. I.1600185. Chemie Meiendors, Germany.
3. Hydroxypropyl methylcellulose 15 cps. (Methocel®E15): Batch No. NC06013T01. Rama Production Co.,Ltd., Thailand.
4. Hydroxypropyl cellulose type H, Batch No. CE-211, Nippon Soda, Japan.
5. Chitosan (85%deacethylation MW 50,000) : Seafresh Chitosan Co., Ltd., Bangkok, Thailand.
6. Chitosan (85%deacethylation MW 100,000) : Seafresh Chitosan Co., Ltd., Bangkok, Thailand.
7. Ethyl cellulose : Batch No. NC06013T01. Rama Chem.,Thailand.
8. Polyethyleneglycol 400 : Batch No. SFA 124/982. Dow Chemical, USA.
9. Propylene Glycol : Batch No. 7879040502. Shell Co.,Ltd. Singapore.
10. Aluminium hydroxide : Batch. No.S4870.B.L.Hua & Co.,Ltd. Thailand.
11. Dicalcium phosphate : Lot. No. 24024. Samyod Co.Ltd.,Thailand.
12. Calcium carbonate : Lot. No. 21052. Fluka, Switzerland.
13. Potassium chloride: Lot. No. TA915536 124. Merck, Germany.
14. Potassium nitrate : Lot. No. A264136 117. Merck, Germany.
15. Potassium dihydrogen orthophosphate : Batch No. F2H145. APS, Australia.
16. Sodium hydroxide pellets : Batch No. 7708MVKK. Mallinckrodt Baker, Maxico.
17. Carbopol 934P NF : Batch No. CC24HBB151, NOVEN, USA.
18. Glacial acetic acid : Batch No. K18049863. Merck, Germany.
19. Methylparaben : Batch No. 406565/1 216000. Fluka, Switzerland.
20. Sodium chloride : Batch No. F2C273, BDH Laboratory Supplied, England.
21. Citric acid : Batch No 0086978, Fisher Scientific, England.
22. Acetonitrile HPLC grade : Batch No. 03010054. Labscan Co.,Ltd. Ireland.
23. Methanol HPLC grade : Batch No. 03041123. Labscan Co.,Ltd. Ireland.

24. Ultrapure Water equipped with filter system (Balson®,Balson Inc., USA).
25. Standard buffer solution : Beckman, USA.

All chemical were of analytical grades or pharmaceutical grades and were used as received.

Equipment

1. Analytical balance : Satorius, model A200s, Satorius Co.,Ltd., Germany.
2. Magnetic stirrer : Heidolph, model MR3001, Germany.
3. pH meter : Thermo Orion, model 201, USA.
4. Sonicator : ELMA Transsonic Digitals, Type TP680DH, Germany.
5. Diffusion cells : Modified from Franz's diffusion cell.
6. Hot plate stirrer : model GeM HS-101, Thailand.
7. High-performance liquid chromatography (HPLC) instrument equipped with the following
    - Liquid chromatograph pump : LC-10AD, Shimadzu, Japan.
    - UV-VIS detector : SPD-10A, Shimadzu, Japan.
    - Recorder : C-R6A chromatopac, Shimadzu, Japan.
    - C-18 column, 250 x 4.6 mm, 5 μm : Hypersil®,BDS, England.
8. Tensile tester : Instron 5565, Instron Corp., England.
9. Ball mill : M.S. FLUID Co.,Ltd. Thailand.
10. Hot air oven : Memmert type BM600, Germany.
11. Micrometer : Teclock Co., Japan.
12. Differential scanning calorimeter : NETZCH DSC 200, NETZSCH-Geratebau, GmbH, Germany.
13. Scanning-electron microscope : JSM-5410LV, Jeol, Japan.
14. X-ray diffractometer : model JDX-8030, Joel, Japan.
15. Fourier transform infrared spectrometer : Model 1760X, Perkin Elmer, USA.

Glassware and Miscellaneous

1. Dialysis membrane : cut off size 12,000-14,000. Lot No. 28H0141, Sigma, Germany.
2. 0.45 μm membrane filter : Waters, USA.
3. Beaker : Pyrex, USA.
4. Cylinder : Pyrex, USA.
5. Test tube : Pyrex, USA.
6. Transfer pipette, Germany.
7. Volumetric flask, Germany.
8. Disposable syringe : Terumo, Thailand.
9. Filter device : Swinnex, Millipore, USA.
10. Aluminium foil : MMP packing, Thailand.
11. Parafilm : American National Can., USA.

Methods

A. Preparation

1. Lidocaine film

Mucosdhesive patches containing 45 mg of lidocaine per 2 cm^2 were prepared. The compositions of formulas are indicated in Tables 2-3. The procedures for the preparation of mucoadhesive patches were as the following:

HPMC E15, HPC-H and Carbopol 934P 5%w/w solution were individually prepared by dissolving the polymer in distilled water. The polymeric solutions were stirred at room temperature overnight in order for complete hydration and swelling. Chitosan was pulverized in a ball mill and passed through 80 mesh screen. Required quantity of chitosan (5% w/w) was gradually dispersed in half of required volume of water. The amount of citric acid giving 3% w/w acid in final solution was dissolved in another part of water and then added into previous dispersion. The polymer solution

was adjusted to weight with water. Then lidocaine was added into the polymeric solutions. The solutions were poured on glass plate (9.0 cm in diameter) and left to stand until the trapped bubbles were removed. The patches were dried for 12 hours or until constant weight in a hot air oven at 45°C. The dried films were stored in desiccator until the time for analysis.

The lidocaine mucoadhesive films were prepared by using ratio of drug : polymer as 2:0.5, 2:1 and 2:2.

The physical characteristics such as transparency, glossiness, flexibility, stickiness and ease to peel off from glass plate were observed. The satisfied formulas would be chosen for further development.

Table 2 The formulations of lidocaine base mucoadhesive patches

Substance (g)	Formulation (g)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Lidocaine base	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
HPMC E15 ($E15_B$)	0.5	1.0	2.0												
HPC-H (HPC_B)				0.5	1.0	2.0									
Chitosan 50,000 (CS_{1B})							0.5	1.0	2.0						
Chitosan 100,000 (CS_{2B})										0.5	1.0	2.0			
Carbopol 934P (CB_B)													0.5	1.0	2.0
Purified water qs.	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.

Table 3 The formulations of lidocaine HCl mucoadhesive patches

Substance (g)	Formulation (g)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Lidocaine HCl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
HPMC E15 ($E15_H$)	0.5	1.0	2.0												
HPC-H (HPC_H)				0.5	1.0	2.0									
Chitosan 50,000 (CS_{1H})							0.5	1.0	2.0						
Chitosan 100,000 (CS_{2H})										0.5	1.0	2.0			
Carbopol 934P (CB_H)													0.5	1.0	2.0
Purified water qs.	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.

2. Preparation of backing

The solvent-based free films were prepared by casting method as previously described. Briefly, ethyl cellulose (EC) as film-forming polymer and triacetin as plasticizer were dissloved in 95% ethanol, forming 1-3% (w/v) solution of EC. Triacetin was added in ratio of 0-3% (w/v) solution. The solution of about 10 ml was cast on glass plates and dried at 45°C for 6 hours.

Table 4 The formulations of backing using ethyl cellulose as polymer

Substances	formulations											
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
Ethyl cellulose (g)	1	1	1	1	2	2	2	2	3	3	3	3
Triacetin (g)	0	1	2	3	0	1	2	3	0	1	2	3
Ethanol qs. (ml)	100	100	100	100	100	100	100	100	100	100	100	100

B = formulation of backing

The physical characteristic such as transparency, glossiness, flexibility, stickiness and ease to peel off from glass plate were observed. The satisfied formulas would be chosen for further development by mixing with inorganic substances which were sieved by 40 mesh such as aluminium hydroxide, dicalcium phosphate, and calcium carbonate in order to make rough surfaced film then pour the drug free film solutions from the selected lidocaine film formula. The formulations are shown in table 5. Backing with drug free film would be determined in term of detachment force between backing and free film. The selected formula would be brought to prepared lidocaine patch.

Table 5 The formulations of backing using ratio of the selected backing and inorganic substance in 0.1-0.4 g per 100 ml of the selected backing solution.

Inorganic substances	Amount of inorganic substances (g/100 ml of the selected backing solution)			
Aluminium hydroxide	0.1	0.2	0.3	0.4
Dicalcium phosphate	0.1	0.2	0.3	0.4
Calcium carbonate	0.1	0.2	0.3	0.4

3. Preparation of lidocaine patches

After the preparation of the backing, lidocaine film from the selected matrix film was prepared, put on the backing and dried at 45°C for 12 hours in order to make as layer. Then using carbopol 934P 1%, 2% and 3% w/v and pour from 4 ml to 10 ml on the matrix film, dried at 45°C until constant weight and the dried patches were evaluated.

B. Evaluation

1. Physical characteristics

The obtained films and backing were observed for transparency, ease to peel off from glass plates, glossiness, flexibility, and stickiness.

2. Thickness measurement

The thickness of the mucoadhesive films were measured by a micrometer. The samples were measured in triplicate. Each sample was measured at five locations as figure 10. The mean and the standard deviation of thickness were calculated.

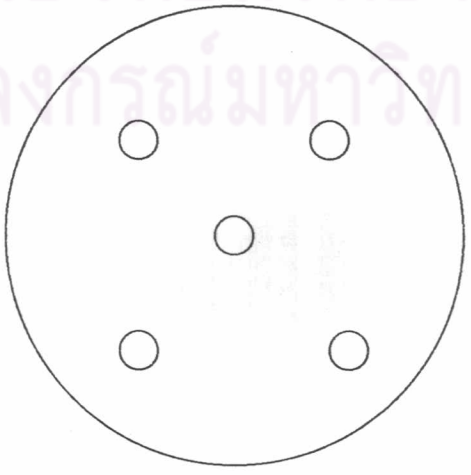

Figure 10 The measured positions of thickness of the films

3. *In vitro* evaluation of mucoadhesive patches

The satisfactory formulas of the patches were chosen and evaluated as follows and compared with commercial product, Dentipatch®.

3.1 Tensile properties

The ultimate tensile strength, modulus of elasticity (Young's modulus) and percentage of strain at point of break of the obtained films were examined by using a universal tensile testing machine equipped with 10N tensile load cell under ASTM standard D882-88. The data of tensile properties were obtained by the following procedure.

The films specimens were cut into small strips (2.00 x 80.00 mm) by using a standard knife. The specimens were left to expose to room humidity for 1 hours before tested. The thickness of each strip was the mean value of five separate measurements taken along the length of the sample by using micrometer. Then the strips were carefully clamped by an upper and lower pneumatic flat-faced grip and were extended by the test machine at speed of 5.0 mm/min until it was ruptured.

The tensile stress was plotted against the strain to give stress-strain curve, and ultimate tensile as well as elongation at break was reported. The mean and standard deviation of the values obtained from six-determinations.

3.2 Adhesive property

3.2.1. Adhesive property between backing and films

The adhesiveness of the films which had both layer of backing and drug free film were measured by fixing the films between the aluminium flat surfaces on an Instron tensile tester equipped with computer integrated data acquisition. The samples were cut into small pieces (1.00 x 1.00 cm). The moving part was raised at a constant speed (20mm/min) and the force required for detachment was recorded. The data were

analyzed using Series IX software (Instron corp.) and were reported as the maximum force required for detachment per cross sectional area.

3.2.2. Mucoadhesive property of lidocaine patches

The patches were cut into small pieces (1.00 x 1.00 cm). Each piece of sample was fixed with the backing side on the aluminium flat surfaces and placed the mucoadhesive side on the surface of mucous membrane of pig intestine which fixed on the aluminium stationary platform then moistened with 100 µl of artificial saliva pH 7.0 and left to swell for 2 min. After a pre-set time (2 min) of contact, the crosshead was raised at a constant speed (20mm/min) and the force required for detachment was recorded. The data were analyzed using Series IX software (Instron corp.) and were reported as the maximum force required for detachment per cross sectional area.

3.3 Content uniformity determination

The content uniformity of lidocaine in the mucoadhesive patches were quantitatively determined by mean of absorption peak area ratio by HPLC method. The standard USP method as follows:

For analysis purpose, the film was cut into ten small pieces (1.00 x 2.00 cm). Each piece accordingly contained 45 mg of lidocaine. They are individually tested for their content uniformity. Each piece was analyzed by dissolving in 100 ml of water in 100-ml volumetric flask and shaken at room temperature until the patch was completely dissolved in water. The obtained solution was taken to measure the absorbance by HPLC. The percentage of active drug within the test films was calculated from the calibration curve.

3.4 Analysis of lidocaine

3.4.1 HPLC chromatographic condition

The chromatographic condition used was adapted from USP24. Its condition was presented as follows:

Column : Hypersil® C18 column (250 x 4.60 mm), 5μm (UK)

Detector : UV detector was set at 254 nm

Flow rate : 1.2 ml/min

Attenuation : 2

Chat speed : 2 cm/min

Injection volume : 20 μl

Internal standard : methyl paraben 1 μg/ml

Mobile phase : 50 ml of glacial acetic acid was mixed with 930 ml of ultrapure water, and then it was adjusted to pH 3.4 with 1N sodium hydroxide. 8 volume of this solution was mixed with 3 volume of acetronitrile. The mobile phase was prepared freshly and filtered through a 0.45 μm membrane filter then degassed by sonication for 30 min prior to use.

3.4.2 Validation of HPLC method

The analytical parameter used for the assay validation were specification, accuracy, precision and linearity (USP24/NF19,1999).

a) Specificity

Under the chromatographic condition used, the peak of lidocaine base and lidocaine HCl had to be completely separated from and be interfered by the peak of other components in the sample. Phosphate buffer and non-active ingredients, including HPMC E15, HPC-H, Chitosan, EC and carbopol 934P were

injected. Chromatograms were evaluated by comparing with the standard solution of lidocaine base and lidocaine HCl.

b) Accuracy

Three sets of standard solutions of lidocaine base and lidocaine HCl having concentrations of 100-300 μg/ml were prepared and injected. The percentage of the analytical recovery of each standard solution was calculated.

c) Precision

Within Run Precision

The within run precision was determined by analyzing three sets of five standard solutions of lidocaine base and lidocaine HCl in the same day. Peak area ratios of lidocaine base and lidocaine HCl to the internal standard were compared and percentage coefficient of variation (%CV) of each concentration were determined.

Between Run Precision

The between run precision was determined by comparing each concentration of lidocaine base and lidocaine HCl standard solutions that were prepared and injected on different days. The percentage coefficient of variation (%CV) of lidocaine base and lidocaine HCl to their internal standard peak area ratios from the three sets of standard solutions having the same concentration were determined.

d) Linearity

Lidocaine base and lidocaine HCl standard solutions ranging from 10 to 400 μg/ml were prepared and analyzed. Linear regression analysis of peak area ratios versus their concentrations was performed.

3.4.3 System suitability

a) Resolution

The resolution was a function of column efficiency and was specified to ensure that lidocaine base and lidocaine HCl was resolved from methyl paraben. The resolution, R was determined by the equation

$$R = \frac{2\,(t_2-t_1)}{W_2+W_1} \quad \text{.....[1]}$$

In which t_1 and t_2 were the retention times of lidocaine base or lidocaine HCl and methly paraben, respectively. W_2 and W_1 were the corresponding widths at the base of peaks obtained by extrapolating the tangent of peak to the baseline.

b) Tailing factor

Tailing factor was performed by collecting data from injected standard solutions. This test was determined by equation

$$T = \frac{W_x}{2f} \quad \text{.....[2]}$$

In which W_x was the width of peak of lidocaine base or lidocaine HCl and methyl paraben, at 5% height, f was the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

3.4.4 Calibration curve of lidocaine base and lidocaine HCl

a) Stock solution

The stock solution of internal standard was prepared by transferring about 20 mg of methyl paraben, accurately weighed, to a 100-ml volumetric flask. Ultrapure water was used to adjust to volume. Then 1 ml of this solution was pipetted and transferred into 100-ml volumetric flask then adjusted to volume with ultrapure water.

The stock solutions of lidocaine base and lidocaine HCl were prepared by transferring about 50 mg of lidocaine, accurately weighed, to a 100-ml volumetric flask. Deionized water was used to adjust to volume. Then 10 ml of this solution was pipetted and transferred into 100-ml volumetric flask. Deionized water was used to adjusted volume.

b) Standard solutions for calibration curve

Standard solutions of lidocaine (100, 200,300, 400, and 500 μg/ml) containing 1 μg/ml of methyl paraben were prepared from stock solution of lidocaine and methyl paraben by diluted and adjusted to volume with ultrapure water.

3.5 The physicochemical characterization

Infrared spectrometry, X-ray diffractometry and differential thermal analysis were used to characterize the substances in the patches.

3.5.1 Infrared spectrometry

Fourier transform infrared spectrophotometry (FT-IR) was used to study the change in the functional groups of the polymers, drug and the mucoadhesive patches. Infrared spectra were examined by using a fourier transform infrared

spectrometer. The obtained films were examined by using KBr disc method. The sample disc was determined by FT-IR spectrometer within the wave numbers of 500-4000 cm^{-1}.

3.5.2 Powder X-ray diffractometry

Powder X-ray diffractometry was used to determine the diffraction angles of substances that showed crystallinity and interplannar spacing of the crystal planes. This mode was used to study the change of crystallinity of polymers and drug after preparation which could explain some physiochemical properties of mucoadhesive patches. The X-ray diffractograms from the patches and the initial powder of lidocaine and polymers were examined by the diffraction method with nickle-filtered CuKα radiation of Jeol diffractometer operated in the ω-2θ scanning mode between 5° and 60° 2θ at 1.50° per second.

3.5.3 Differential scanning calorimetry

Differential scanning calorimetry was used to determine the thermograms of polymers and drug. The differences in thermal energy patterns between the original substances and their products were evaluated. This method was used to study interaction between components after preparation. About 2 mg of original powder or 5 mg of products of each sample were accurately weighed into the DSC pan. Then it was crimped with the sealed pan and placed in the equipment beside the reference pan made by the same method except without powder. The thermal runs were controlled at a heating rate of 10°C per minute and in the range of 30°C to 200°C, sensitivity ± 50 μv and chart speed 10 mm/min in static air atmosphere.

3.6 Moisture sorption and swelling property

3.6.1 Moisture sorption study

The moisture sorption of mucoadhesive patches of 1.00 x 2.00 cm small strip were determined. The original dry weight (W_0) of the strips at room temperature (about 25°C) was obtained after keeping them for 24 hours in a desiccator which filled with silica gel. Then they were placed inside desiccators containing saturated solutions of magnesium nitrate (53%RH), sodium chloride (75%RH), potassium chloride (84%RH) and stored at 25°C (Umprayn and Mendes, 1987). At appropriate time intervals (1, 3, 5, and 7 days), the patches were taken out, and weighed immediately (W_t). The percentages of moisture sorption of the patches were calculated by the following equation.

$$\% \text{ moisture sorption} = \frac{(W_t - W_0)}{W_0} \times 100\% \quad \text{.....[3]}$$

where W_0 is the original dry weight of the strip patches, W_t is the weight of the strip patches at time t. The measurement was made in triplicate.

3.6.2 Swelling measurement

The mucoadhesive patches were cut into 1.00 x 2.00 cm small strips. The strips were determined the initial thickness by using micrometer at room temperature (about 25°C) by keeping them in the desiccator which filled with silica gel for 24 hours and calculated the initial volume (V_0). Then they were placed inside desiccators containing saturated solutions of sodium chloride (75%RH), potassium chloride (84%RH), and potassium nitrate (94%RH) and stored at 25°C. At appropriate time intervals (1, 3, 5, and 7 day), the patches were taken out, and the size (wide and length) and thickness of the patches was measured immediately and then the volume (V_t) was calculated. The percentage of swelling was calculated by the following equation.

$$\% \text{ swelling} = \frac{(V_t - V_0)}{V_0} \times 100\% \quad \text{.....[4]}$$

where V_0 is the original volume of the strip films, V_t is the volume of the strip films at time t. The measurement was made in triplicate.

3.7 In vitro drug release and penetration from mucoadhesive patches

The release and penetration of drug from mucoadhesive patchess were determined using modified Franz diffusion cells (Figure 11). The internal diameter of each cell was 1.70 cm, corresponding to an effective permeable surface area of 2.27 cm^2. The receptor compartment contained about 13.5 ml of pH 6.8 phosphate buffer as penetration medium.

The mucoadhesive patch was clamped between the donor and the receptor compartments with dialysis membrane for penetration study (Senel et al., 2000) or without dialysis membrane for release study. In case of drug penetration through dialysis membrane, The mucoadhesive film was placed directly onto the dialysis membrane. The dialysis membranes were pretreated by immersing in deionized water at room temperature for overnight and then rinsing with 80°C of deionized water for 2 min in order to wash off any water soluble contaminates. After that, they were soaked in phosphate buffer pH 6.8 until used. A small magnetic stirring bar (4 x 7 mm) was placed in the receptor compartment and rotated at 750 rpm. The diffusion cell (capacity 14 ml) was filled with phosphate buffer solution pH 6.8 until it reached to the top level of receiver chamber on which the dialysis membrane or mucoadhesive film was placed, leaving no air bubbles in the chamber. The temperature of the assembled diffusion cell was maintained at 37±1°C by the means of circulating water jacket connected to a constant temperature water bath. A portion of the receiver medium (10 ml each) was withdrawn through at predetermined time interval. The same volume of the solution withdrawn was returned to the chamber at each withdrawal. The amount

of drug released or penetrated was assayed by the HPLC method. The triplicate determinations of each of sample were measured.

Saturated solution of lidocaine was determined for drug penetration through dialysis membrane in order to compare drug penetration profiles of solution and mucoadhesive patches.

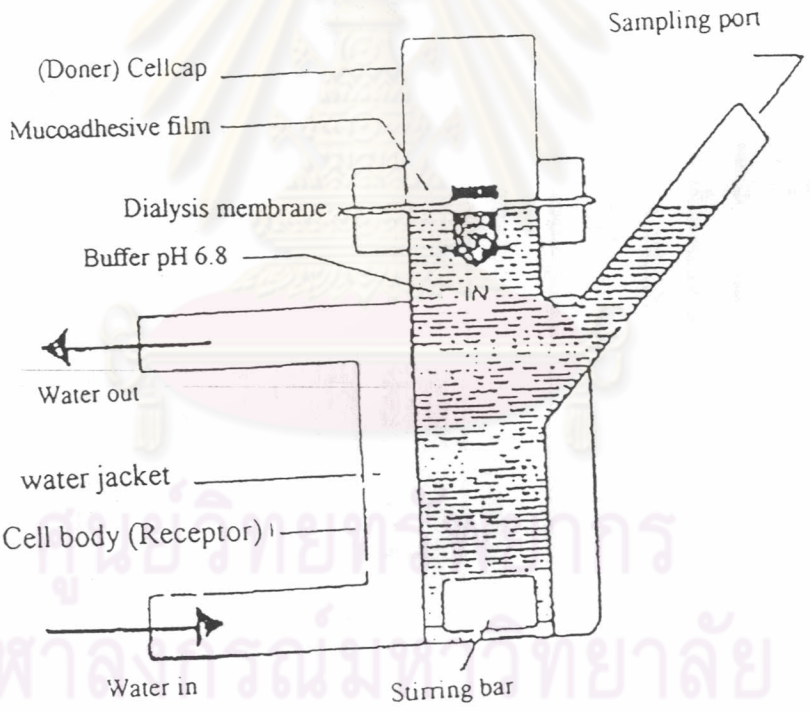

Figure 11 Schematic diagram of the apparatus for the in vitro release and penetration studies

3.8 Stability of mucoadhesive patches

All patches were kept in aluminium foil and sealed with polypropylene tape in order to prevent the patches from light and moisture as shown in figure 12.

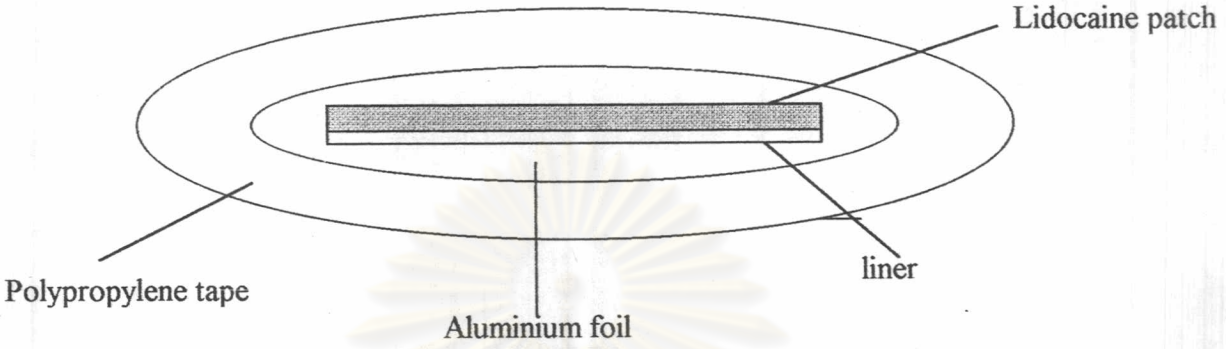

Figure 12 Diagram of packaging of the lidocaine patch

The stability of this study was followed by the guideline of the Thai FDA. The suggested accelerated study could be tested on the conditions as in table 6.

Table 6 The stability study in accelerated conditions guideline of the Thai FDA.

The collected sample temperature (°C)	%RH	Time (months)	Frequency to collect the sample (months)
45	75	4	0,1,2,3,4 or
40	75	6	0,1,3,6 or
50	75	3	0,1,2,3

During the stability test at the condition of 45°C 75%RH, 3 samples of each patches were brought to determine the content by using HPLC method at 0, 1, 2, 3 and 4 months. After 4 months, all patches were also tested on an X-ray diffractometer.