CHAPTER II

MATERIALS AND METHODS

1. Materials

1.1 Methyl salicylate USP (The government Pharmaceutical Organization, Bangkok Metropolis, Thailand)

1.2 Potassium hydroxide AR (BDH)

1.3 Ortho-phosphoric acid AR (BDH)

1.4 Hydrochloric acid AR (BDH)

1.5 Beeswax USP (The Government Pharmaceutical Organization, Bangkok Metropolis, Thailand)

1.6 Banana wax (TISTR)

1.7 Carnauba wax USP

1.8 Ricebran wax (TISTR)

1.9 Sugarcanewax (TISTR)

1.10 Theobroma oil USP

1.11 Calcium chloride AR (BDH)

1.12 Sodium bicarbonate AR (BDH)

1.13 Sodium chloride AR (BDH)

1.14 Potassium chloride AR (BDH)

1.15 Ferric nitrate AR (BDH)

2. Equipments

2.1 Spectrophotometer (Beckman)

2.2 Cellulose membrane (Cellulose dialyzer tubing Arther H Thomas Co)

2.3 Electric mixer (Virtis)

2.4 Constant temperature water bath (Colora)

2.5 Single pan balance (Sauter)

3. Methodology

3.1 Preparation of the standard curve (12)

A Sample 0.1000 gm. of salicylic acid, previously dried 100°C for 1 hour, was weighed and dissolved in 10 ml. of ethanol and diluted to exactly 500 ml. with water. Aliquots of 5, 10, 15, 20 and 25 ml. were placed in 100 ml. volumetric flasks, to each aliquot, 5 ml. of 1% nitric acid solution containing 1% ferric nitrate was added, as the color developer, and the solutions were diluted to volume with water to make solutions of 0.001, 0.002, 0.003, 0.004 and 0.005% concentration respectively. The resultant solutions would be at the optimum pH of between 5 and 6. The absorbances of the solutions were read on the spectrophotometer at 525 nm, versus distilled water, as a controlling blank. The absorbance results were plotted versus percentage concentrations (Figure 5).

This standard curve was used for the Me. Sal. determination for this project study.

3.2 Proparation of Ointment.

A wax, under the study , was melt at its melting point temperature on water bath, Me. Sal. is added into the molten wax, stir until thoroughly mix is obtained, continue stir until congeal. The ointment of each wax have been made to contain 25%, 50% and 75% w/w Me. Sal.

3.3 The study on Me. Sal. releasing property from ointment.

3.3.1 In vitro

The diffusion cell technique method developed by Bottari, F. et al (4) have been used, the diagram of the diffusion cell was shown in figure 2.

The cellulose membrane (B) has been previously soaked in physiological fluid (10) for 12 hours.

The soak membrane was fixed on the diffusion cell A.

Ring A-1 was then fixed on membrane B.

The testing ointment was filled into the cell form by ring A-1 and membrane B. The excess ointment in ring A-1 was removed by the spatula.

The membrane B-1 previously soaked in physiological fluid was put over ring A-1 on the surface of the ointment, care should be made to avoid air entrap between the ointment and cellulose membrane surface.

Ring A-2 was then put on cellulose membrane B-1

Four knots were then fixed tightly on the screwing to form a diffusion cell ready for releasing experiment.

Immersed the diffusion cell into 200 ml. physiological fluid in a beaker and then the beaker was immersed in a circulating constant temperature water bath as shown in figure 3.

The circulating physiological fluid in the beaker was controlled by electric stirrer at medium speed.

25 ml. of physiological fluid under experiment was pipetted every 15 minutes interval for one and a half hours and replace at once with 25 ml. prewarmed physiological fluid ($37^{o}C$).

The withdrawal 25 ml. physiological fluid was chemically assay for salicylic acid content by the method described by T. Higuchi (12).

3.3.2 In vivo

The method, as described by Wurster and Kramer (8) have been employed. In order to maintain a fixed boundary around a measured area of skin surface and to expose the skin to an excess of the ointments for prolonged periods of time, two stainless steel cells (5X10X1 cm/cell) which could be affixed to both forearms were designed (Figure 4). 1.5 gm. of the ointments were applied on each volunteer's forearm covered approximately 50 cm^2 of the skin, then covered with stainless steel cell for eight hours. 3.0 gm. of the ointments were applied on each volunteer, on both forearms. At the end of eight hours the cells were removed and the area cleaned with water and soap. The urine sample were collected at 2, 4, 6, 8, 12 and 24 hours after the ointment was applied. At each time, the volume of the urine excreted was also measured.

The analytical procedure employed to determine total urine salicylate was carried out, using the method developed by Wurster and Kramer (8). The method consisted of the acid hydrolysis of the salicylate conjugates with subsequent steam distillation of the free salicylic acid. A mixture containing 250 ml. of the urine sample and 5 ml. of phosphoric acid was concentrated until the temperature reached 120°C. Steam distillation was then initiated and the temperature increased to 140°C. Continuous steam distillation was carried out until the distillate was shown to be devoid of salicylic acid when

tested with ferric nitrate test solution (5% ferric nitrate in 0.1 N nitric acid)

To a 50 ml. sample of the distillate ferric nitrate tese solution (1 ml.) was added to develope the characteristic color of the iron-salicylate complex. The absorbance of this solutions was determined at 525 nm. using spectrophotometer. A solution containing an equivalent amount of the test solution in distilled water was imployed as the blank.

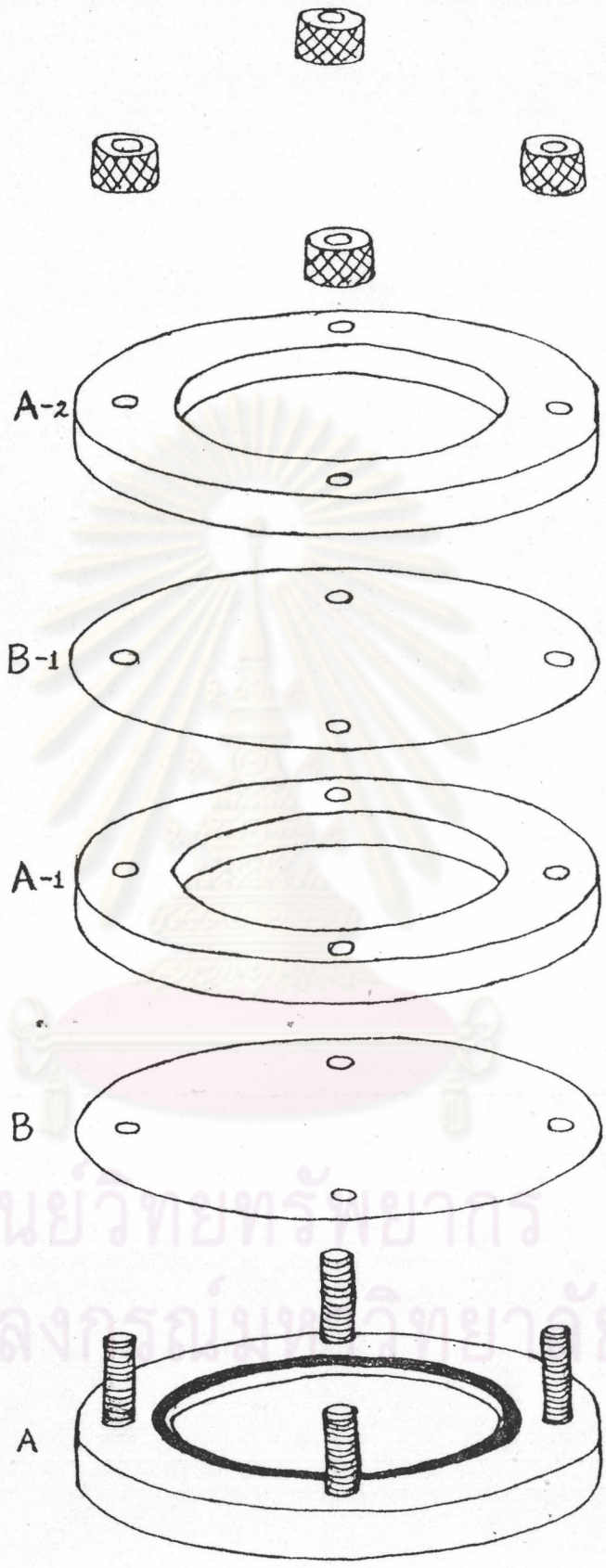

Figure 2 Diffusion cell used for releasing experiments.

A : Stainless steel cell body and plate

B : Cellulose membrane.

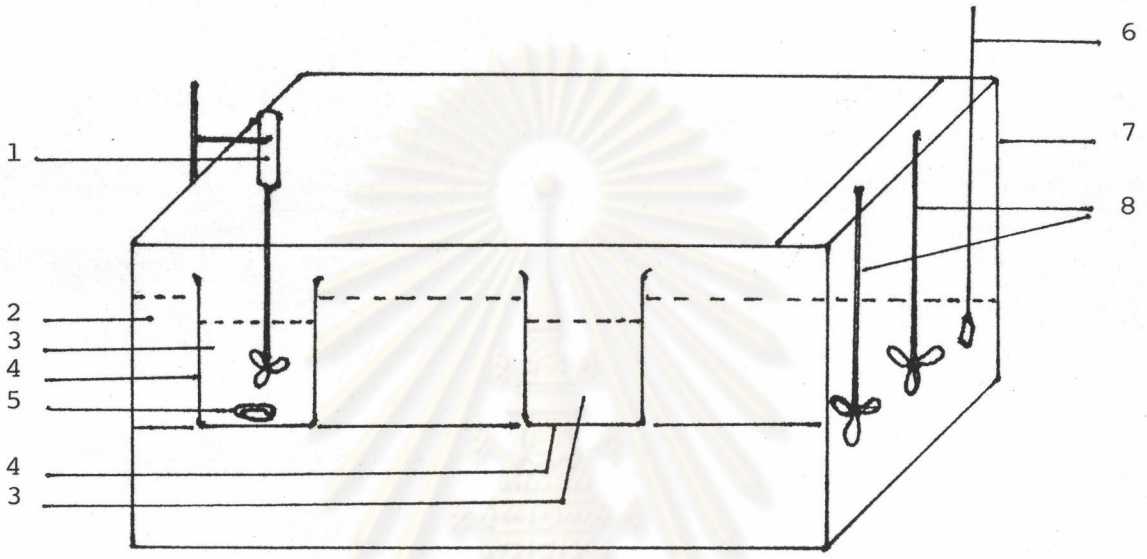

Figure 3 Diffusion cell for in vitro study on Me. Sal. releasing rate.

1. Electric mixer
2. Water (37°C)
3. Physiological fluid
4. Beaker
5. Diffusion cell
6. Thermometer
7. The circulating constant temperature water bath
8. Electric fan

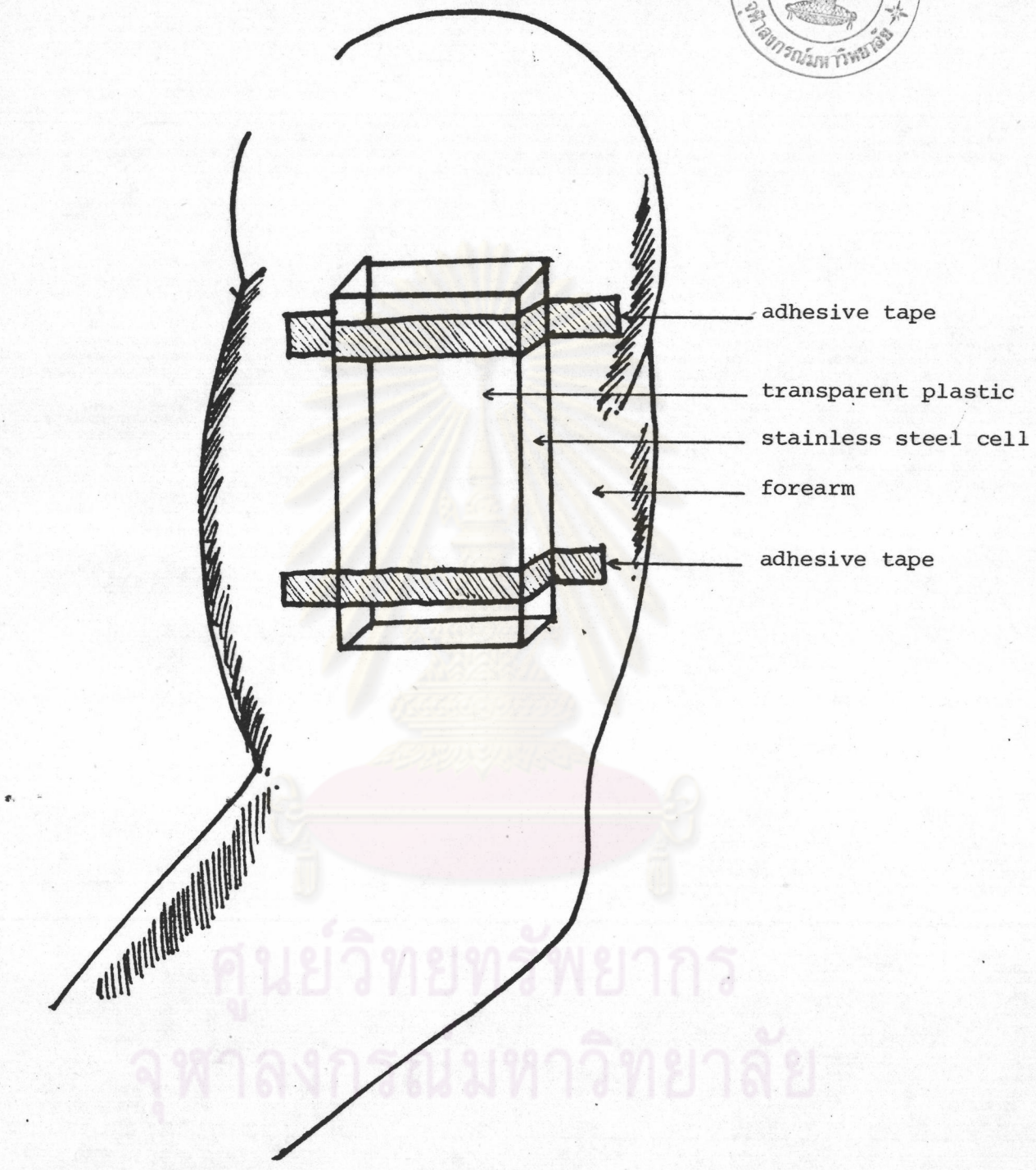

Figure 4 Absorption cell (5X10X1 cm.)