CHAPTER III

EXPERIMENTAL

Materials:

Miconazole nitrate, Batch No. H 002, Yung Zip, Taiwan.

Sodium carboxymethylcellulose medium viscosity, S. Tong Chemicals Co., Ltd.

Sodium carboxymethylcellulose high viscosity, S. Tong Chemicals Co., Ltd.

Methylcellulose 1500, S. Tong Chemicals Co., Ltd.

Methylcellulose 4000, S. Tong Chemicals Co., Ltd.

Hydroxypropylmethylcellulose 4000, S. Tong Chemicals Co., Ltd.

Carbopol 934, S. Tong Chemical Co., Ltd.

Clotrimazole, Batch No. 91103, with the supplement from Greater Pharma Co., Ltd.

Ammonium dihydrogenphosphate, Carlo Erba Co., Ltd.

Mucin, Sigma Co., Ltd.

Sodium sulfide, with the supplement from the Faculty of Science.

Magnesium chloride, with the supplement from the Faculty of Science.

Potassium chloride, E. Merck Co., Ltd.

Sodium pyrrophosphate, Mallinckrodt Co., Ltd.

Sodium hydrogenphosphate, E. Merck Co., Ltd.

Calcium chloride, E. Merck Co., Ltd.

Urea, Srichan Co., Ltd.

Ethanal, E. Merck Co., Ltd.

Methanol, E. Merck Co., Ltd.

All chemicals were analytical or pharmaceutical grades and were used as received.

Equipments:

Double-beam spectrophotometer, model U-2000(Hitachi,Japan)

Hot air oven (Memmert^{R}, Germany)

High performance liquid chromatography (HPLC) equipped with

Absorbance detector (Water, USA)

Constant flow pump (Water 510, USA)

Integrator (Water 745 B, USA)

C 18 Column, 4.6 mm id x 25 cm, part No: 839540, S5 ODS2 (Spherisorb^{R}, UK)

Tension balance for surface & interfacial tension measurement (White Elec inst. Co., Ltd., England)

Analytical balance (Satorius 1615 MP, Germany)

Sonicator (Bransonic^{R} 221, USA)

Modified Franz diffusion cell apparatus, (Atlantic Pharmaceutical Co.,Ltd, Thailand)

Methods:

1. **Preparation of Miconazole Mucoadhesive Patches.**

Mucoadhesive patches containing 2% w/w miconazole were prepared by the contact plate method described in 1.1-1.5. Preliminary studies indicated some bioadhesive polymers that could be prepared as the patches which included HPMC, SCMC, and MC. The inclusion of CP934 at the ratio of 1:9 (CP934:the cellulose derivatives) was also possible to prepare the patches. The formulas are shown in tables 1 and 2. The procedures for preparing mucoadhesive patches were as follows:

1.1 The polymer was dispersed in distilled water and the dispersion was left at room temperature for 2-12 h in order that the polymer would completely hydrate and swell.

1.2 Miconazole in ethanol was dissolved.

1.3 Miconazole solution was added into the polymer dispersion and the mixture was stirred gently in order to prevent air entrapping. The mixture was left at room temperature until the air bubbles were removed.

1.4 The patch was prepared by pouring 50 g of the mixture solution on a flat surface of a glass plate.

Table 1: Composition of miconazole mucoadhesive patch formulas using single polymers.

Composition	Concentration in solution (%w/w)	Concentration in dried patch (%w/w)
Miconazole	0.02	2
Polymer	0.98	98
Ethanol	30.0	-
Purified water	69.0	-

Formulation # 1: polymer = SCMC MV.

Formulation # 3: polymer = SCMC HV.

Formulation # 4: polymer = MC 1500.

Formulation # 6: polymer = MC 4000.

Formulation # 8: polymer = HPMC.

Table 2: Composition of miconazole mucoadhesive patch formulas using combined polymers.

Composition	Concentration in solution (%w/w)	Concentration in dried patch (%w/w)
Miconazole	0.02	2
Polymer	0.882	88.2
CP 934	0.098	9.8
Ethanol	30.0	-
Purified water	69.0	-

Formulation # 2: polymer = SCMC MV.

Formulation # 5: polymer = MC 1500.

Formulation # 7: polymer = MC 4000.

Formulation # 9: polymer = HPMC.

1.5 The glass plate was placed in a hot air-oven at 60°C. The patch was completely dried in the oven. After the solvents which were ethanol and water had been evaporated, the final weight was about 0.5 g and the thickness of patches were about 0.07 mm-0.09 mm.

2. *In Vitro* Mucoadhesion Studies.

In this investigation, the method was modified from Smart's method (1991). This method was composed of a surface and an interfacial tension torsion balance used for surface tension determination and consisted of a glass plate suspended from a torsion balance (figure 18). A 50 ml glass cup (6.0 cm id x 3.0 cm depth) was used to fill an artificial saliva.

2.1 Zero adjustment: The large index pointer (B) was rotated using the ebonite handle until it was set accurately at zero. The balance beam was unlocked and the knurled disc (A) was rotated. The small beam pointer (C) on the right hand side of the dial should then be at the zero point at the centre of the short beam scale. Should this not be so, then the balance could be reset to zero by means of the knurled "ZERO ADJUSTER" knob at the rear of the balance case. The balance was now ready for use.

2.2 Measurement of adhesive force: The balance was set up using the thicker of the two extension hooks from which the glass test plate should be suspended. The balance was adjusted to zero. A glass cup containing the artificial saliva was placed on a platform that could be lowered. The mucoadhesive patch (5 mm wide x 40 mm long) was adhered to the plate.

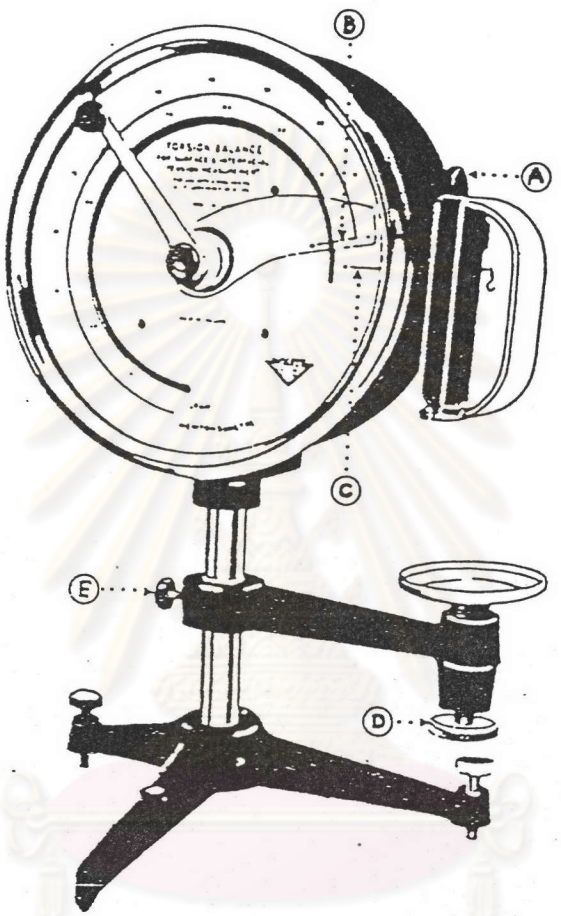

Figure 18: Tension balance for determining bioadhesive tensile strength.

The platform was raised until the plate had penetrated the artificial saliva. The plate had been left in contact with the artificial saliva for 7 min before the platform was lowered. The height of the artificial saliva was gradually lowered by means of the adjusting screw (D) and the index pointer (B) was

moved at the same time in an anti-clockwise direction so as to maintain the bean pointer (C) at zero. The value indicated by the index pointer (B) was read at the moment when the glass plate was exactly over the artificial saliva. The formula of the artifical saliva was modified from Fusayama, Katayory and Nomoto (1963) as follows.

Artificial Saliva

Na_2S	0.160	mg
$Na_4P_2O_7$	0.191	mg
Mucin	400.000	mg
Na_2HPO_4	60.000	mg
$CaCl_2$	60.000	mg
KCl	40.000	mg
NaCl	39.832	mg
$MgCl_2$	0.136	mg
$CO(NH_2)_2$	100.000	mg
Distilled water qs.	100	ml

As a standard adhesive force, the clean plate was tested before and after adhering with the test materials, and the mean of the three measurements was determined. Then, three patch samples were tested and the mean adhesive force was then expressed as a percentage of the clean plate force.

3. *In Vitro* Release of Miconazole from Mucoadhesive Patches.

A test preparation was adhered onto the lower surface of a support which placed on a receiver chamber of modified Franz diffusion cell using a metal clamp. The receiving compartment contained artificial saliva (in 2.2) not including mucin. It was controlled at 37 ^{o}C using a circulating water jacket which was connected to a constant temperature water bath. The receiving solution was kept well stirred at 300 $\pm$ 5 rpm by a magnetic stirrer throughout the time of release studies. The removal of any air bubbles formed under the preparation has been necessary before the experiment was started. Each formulation was tested in triplicate.

Periodically the receiving solution was sampled via the side arm sampling port of diffusion cell. The entire receiving solution was removed using a syringe fitted with a piece of flexible tubing and the receiver compartment was replaced with fresh artificial saliva and the run was continued. Approximately three minutes were required for the sample to be removed and filled with fresh artificial saliva.

All receiver solutions taken were analyzed using the HPLC technique. Freshly prepared standard solutions were injected regularly during the analysis of each release experiment. The drug concentration was then determined using the calibration curve. The amount release was calculated by multiplying the miconazole concentration by the receiving volume. The receiving volumes varied between 5.7 and 6.1 ml.

4. HPLC Analysis.

The liquid chromatography was composed of a constant flow pump (Water 510) a variable wavelength UV absorption detector (Water 484), and an integrator (Water 745B). Injections were made using a 20-mcl constant-volume injector valve. The chromatography was operated at a flow rate of 1 ml/min. From the spectrum of miconazole was obtained using a Hitachi model U-2000 (figure 19). Therefore, the eluent was monitored spectrophotometrically at 214 nm. The column used was Spherisorb^{R} which was a C-18 column with a diameter of 4.6 mm. and a length of 25 cm. The mobile phase was a mixture of 90% v/v methanol and 10% v/v 0.05 M ammonium dihydrogenphosphate. Clotrimazole solution (0.472 mcg/ml) was

Figure 19: Absorption spectrum of 0.20 mg% miconazole.

used as an internal standard. The peak area ratio was used to determine miconazole concentration. The retention time of clotrimazole and miconazole were 5.5 min and 8.4 min, respectively. The run time per sample was ten minutes.

4.1 Preparation of internal standards: One hundred and twenty mg of clotrimazole was acculately weighed into a 50 ml volumetric flask. Methanol was added and swirled until clotrimazole dissolved completely. One ml of this solution was transferred to another 50 ml volumetric flask and was diluted with methanol to volume. Four ml of this soluton was transferred to a 100 ml volumetric flask and was further diluted with methanol to volume so that the final concentration of clotrimazole was 1.92 mcg/ml.

4.2 Preparation of standard solutions: One hundred and twenty mg of miconazole nitrate was accurately weighed into a 50 ml volumetric flask. Methanol was added and the mixture was swirled until miconazole dissolved completely. This stock solution had a final concentration of 2.4 mg/ml (S1). One ml of the miconazole stock solution (S1) was pipetted and transferred to a 50 ml volumetric flask. It was then diluted with 90:10 methanol:0.05 M ammonium dihydrogenphosphate mixture to volume so that the final concentration of miconazole was 48 mcg/ml (S2). S3 and S4 were prepared in similar manners using 90:10 methanol:0.05 M ammonium dihydrogenphosphate as their solvent mixture. Details of S2, S3 and S4 are shown in table 3.

Table 3: Details of S2, S3 and S4 solutions.

Stock solution	Stock solution obtained volume (ml)	Final volume (ml)	Final concentration (mcg/ml)
S2	S1:1.0	50	48
S3	S2:1.0	10	4.8
S4	S3:1.0	10	0.48

Composition details of standard solutions are presented in table 4. The final volume was adjusted using 90: 10 methanol: 0.05 M ammonium dihydrogenphosphate.

Table 4: Details of miconazole standard solutions.

Standard #	Stock solution obtained volume (ml)	Internal standard (ml)	Final volume (ml)	Final concentration (mcg/ml)
1	S4:0.6	1.0	4	0.072
2	S4:1.0	1.0	4	0.120
3	S4:3.0	1.0	4	0.360
4	S3:0.6	1.0	4	0.720
5	S3:1.0	1.0	4	1.200
6	S3:3.0	1.0	4	3.600
7	S2:0.6	1.0	4	7.200

4.3 Preparation of sample solutions: One ml of receiving fluid was pipetted and transferred to a test tube containing 1.0 ml of the internal standard. Two ml of the 90:10 methanol:0.05 M ammonium dihydrogenphosphate mixture was pipetted and transferred to the test tube so that final volume of sample solution was 4 ml.

5. Stability Studies of Miconazole Mucoadhesive Patches.

The test preparations were stored in amber glass vials, which tighthy sealed with rubber closures and aluminium caps at 40 °C, 90% RH for three months. For maintaining the specified relative humidity in closed chambers, a saturated salt solution was used. The saturated solution of sodium chloride gives 74.8 % RH and 74.7 % RH at 35 °C and 45 °C, respectively (Umprayn,and Mendes, 1987). This study the sodium chloride satulated solution was placed in a desiccator at 40 °C until the water vapour pressure over the system became constant and then the glass vials of the preparations were kept in the desiccator. Before and after storage, all preparations were determined for their % labelled amount and % remaining of miconazole, respectively, using the analytical method in 4. Triplicate samples of each preparation were prepared and tested as follows.

Preparation of samples: Fifteen mg of a sample was accurately weighed in a 25 ml volumetric flask. Methanol:0.05 M ammonium dihydrogenphosphate (90:10) mixture was added and left at room temperature for 12 h in order to let the sample swell and miconazole dissolve completely. The solubility of miconazole was enhanced by immersing the flask in the ultrasonic vibration bath for 1 h. This sample was filtered through a 0.45μm membrane filter to obtain a clear solution. One ml of the solution was pipetted and transferred to a test tube containing 1.0 ml of internal standard

and 2.0 ml of the methanol:0.05 M ammonium dihydrogenphosphate (90:10) mixture.

6. *In Vivo* Resident Time on Buccal Mucosa Studies.

6.1 Test preparations: Six preparations of miconazole mucoadhesive patches different in their polymers, release rates, adhesive forces and stability properties were selected. These formulations showed slow release rate, high adhesive force and good stability.

6.2 Subjects: Thirteen healthy male and female volunteers participated in the study. They were 20 to 31 years old and they had no pathological or physiological disorder in oral cavity.

6.3 Drug administration: Prior to applying a miconazole patch of 2.5 cm wide x 2.5 cm long, all subjects had cleaned their mouth before a test preparation was adhered to their buccal mucosa. No food was allowed until the patch dissolved completely from the buccal cavity. The resident time of mucosal adhesion was the time span required until the mucoadhesive patch completely lost its adhesive contact with the mucosa.

6.4 Experimental design: The study was conducted in a crossover design. Each subject received the preparation in a randomized order with more than 2 h between each administration as shown in table 5.

Table 5: Oder of the miconazole mucoadhesive patches for each volunteer.

Volunteer #	Formulations*
1	C B E D F A
2	A C B D E F
3	F A E C D B
4	A C B D E F
5	B F A E C D
6	D F C E B A
7	C F B A E D
8	A F B E C D
9	F D B A C E
10	A E D C F B
11	F C A B D B
12	B F A D E C
13	B F E C D A

* A = Formulation #3 (SCMC HV)

B = Formulation #4 (MC 1500)

C = Formulation #6 (MC 4000)

D = Formulation #7 (MC 4000 + CP 934)

E = Formulation #8 (HPMC)

F = Formulation #9 (HPMC + CP 934)