CHAPTER II

EXPERIMENTAL

Materials.

Piroxicam Batch No. 90280, with the supplement from Siam Pharmaceuticals.

Tenoxicam, with the supplement from Siam Pharmaceuticals.

Pluronic F-127, BASF Wyandotte Corporation.

Carbopol - 940, BF Goodrich.

Hydroxyethyl Cellulose, S. Tong Chemicals Co., Ltd.

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

Triethanolamine, Viddhayasom.

Propylene Glycol, Viddhayasom.

Isopropyl Alcohol AR., E. Merck.

Tween 20, ICI.

Brij 30, ICI.

Silastic® sheetings, 8"x6"x0.005", Dow Corning.

Pig skin, Local farm in Nakorn Patom.

Methanol AR., E. Merck.

Monobasic potassium phosphate AR., E. Merck.

Disodium hydrogen phosphate AR., E. Merck.

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

Equipments.

Modified Franz Diffusion Cell Apparatus, Atlantic Pharmaceutical Co., Ltd.

Haake Viscometer Model RC20, RV20, Haake Mess-Technik GmbH U., Germany.

pH-Meter Model SA 520, Orion., USA.

Analytical Balance, H 51 AR, Mettler.

Spectrophotometer, Spectronic 2000, Bausch & Lomb., USA.

Shaker, Edmund Buhler., Germany.

High Performance Liquid Chromatography (HPLC) equipped with
an absorbance detector, FLD-1, Shimadzu
an injector, Rheodyne injector
a constant flow pump, LC-3A, Shimadzu
an integrator, C-R1A, Shimadzu
a Lychro CART® C18 column (4 mm. ID x 12.5 cm.)., E. Merck.

Methods.

1. Approximation of Piroxicam Solubilities in pH 7.4 Phosphate Buffer Solution and Normal Saline Solution at Ambient Temperature.

An excessive amount of piroxicam in 15.0 ml. of pH 7.4 phosphate buffer or 15.0 ml. of normal saline solution contained in a 25.0 ml. volumetric flask was shaken by a shaker for 48 hr. at ambient temperature. The saturated concentrations of piroxicam in both solvents were determined using a calibration curve. Absorbances of all solutions were measured spectrophotometrically at a wavelength of 355 nm (by UV scanning).

Note : Compositions of pH 7.4 phosphate buffer.
A : Monobasic potassium phosphate $\frac{1}{15}$ Molar
B : Disodium hydrogen phosphate $\frac{1}{15}$ Molar

To prepare pH 7.4 phosphate buffer 100 ml., place 19.7 ml. of the monobasic potassium phosphate solution (A) in a 100 ml. volumetric flask, add the disodium hydrogen phosphate solution to volume.

2. Preparation of Samples.

Details of all formulations prepared are displayed in Table 3. Various synthetic gelling agents used in different concentrations (pluronic F-68 and pluronic F-127, 18-30%; carbopol 934 and carbopol 940, 0.5-1%; hydroxypropyl methylcellulose, 1-3.5%; hydroxyethyl cellulose, 1-3% and polyvinyl alcohol, 5-10%) had been preliminary studied as gel bases. And the following gelling agents were selected : 20 % pluronic F-127, 1 % carbopol - 940, 3.5 % hydroxypropyl methylcellulose, and 2.5 % hydroxyethyl cellulose. The concentrations of the gelling agents selected would give gel bases with about the same viscosities except pluronic F - 127. A stable pluronic F - 127 gel base would form at the concentration of at least 20 % which was more viscous than the others.

The pH and viscosity of a formulation were important factors of skin penetration characteristics. So, in this study all preparations were adjusted to about the same pH. The preparations not containing any additives were also adjusted to about the same viscosity.

Table 3 : Composition of piroxicam gels.

INGREDIENT	FORMULATION (% W/W)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Piroxicam	2	2	2	2	2	2	2	2	2	2	2	2
Triethanolamine	6	6	6	6	6	6	6	6	6	6	6	6
Pluronic F-127	20	-	-	-	-	-	-	-	-	-	-	-
Carbopol-940	-	1	-	-	1	1	1	1	1	1	1	1
HPMC*	-	-	3.50	-	-	-	-	-	-	-	-	-
HEC**	-	-	-	2.50	-	-	-	-	-	-	-	-
Isopropyl Alcohol	-	-	-	-	10	20	-	-	-	-	-	-
Propylene Glycol	-	-	-	-	-	-	5	10	-	-	-	-
Tween 20	-	-	-	-	-	-	-	-	0.50	1	-	-
Brij 30	-	-	-	-	-	-	-	-	-	-	0.50	1
Water qs. to	100	100	100	100	100	100	100	100	100	100	100	100

* HPMC = Hydroxypropyl methylcellulose
** HEC = Hydroxyethyl cellulose

2.1 Preparation of Piroxicam in Various Gel Bases.

2.1.1 Piroxicam in Pluronic F - 127 (Formulation I).

A gel base of 20 % w/w pluronic F - 127 (PF - 127) was prepared by the cold process (Schmolka, 1972). A weighed amount of PF - 127 was slowly added to distilled water (5-10°C) over a period of 2-3 min. with gentle mixing. The PF - 127 was then allowed to hydrate and disperse overnight in a refrigerator. With time, a clear and viscous solution was formed.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The solution was then added to the cold PF - 127 gel base and the system was incubated in the gel state at room temperature until clarity was restored. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.1.2 Piroxicam in Carbopol - 940 (Formulation II).

Carbopol 940 (1.0 % W/W) was dispersed in water (about 1/3 of the formula) with continuous stirring. The uniform solution was then neutralized with triethanolamine to pH ~ 7.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The piroxicam solution was then added to the carbopol - 940 gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.1.3 Piroxicam in Hydroxypropyl methylcellulose (Formulation III).

Hydroxypropyl methylcellulose (HPMC) (3.5 % W/W) was gradually dispersed in hot water (80-90°C) (about 1/5 to 1/3 of the formula) with continuous stirring. Cold water (4°C) was added to the uniform solution immediately to form a clear gel.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The piroxicam solution was then added to the HPMC gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.1.4 Piroxicam in Hydroxyethyl cellulose (Formulation IV).

Hydroxyethyl cellulose (HEC) (2.5 % W/W) was gradually dispersed in water (~ 65°C) (about 2/3 of the formula) with continuous stirring until it was uniformly dispersed.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The piroxicam solution was then added to the HEC gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

pH's and viscosities of all preparations prepared were determined as described in method no. 3 (p. 32) and no. 4 (p. 33), respectively. The steady-state flux of piroxicam from these four gel preparations through silastic® and pig skin were investigated using modified-Franz diffusion cells (method no. 5, p. 33).

The results obtained from in-vitro diffusion studies demonstrated that piroxicam in 1.0 % carbopol - 940 gel base yielded the highest flux through both silastic® and pig skin. Therefore, 1.0 % carbopol - 940 gel base was selected for further studies.

2.2 Preparation of Piroxicam in Carbopol - 940 Gel Base With Various Additives.

2.2.1 Piroxicam in Carbopol - 940 Gel Base Containing Isopropyl Alcohol (Formulation V and VI).

Carbopol - 940 (1.0 % W/W) was dispersed in 10 or 20 % isopropyl alcohol/water mixture with continuous stirring. The uniform solution was then neutralized with triethanolamine to pH ~ 7.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The piroxicam solution was then added to the gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.2.2 Piroxicam in Carbopol - 940 Gel Base Containing Propylene Glycol (Formation VII and VIII).

Carbopol - 940 (1.0 % W/W) was dispersed in 5 or 10 % propylene glycol/water mixture with continuous stirring. The uniform solution was then neutralized with triethanolamine to pH ~ 7.

Piroxicam (2 g) was dissolved in about 20 ml. of water with the aid of triethanolamine. The piroxicam solution was then added to the gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.2.3 Piroxicam in Carbopol - 940 Gel Base Containing Tween 20 (Formulation IX and X).

Carbopol - 940 (1.0 % W/W) was dispersed in water (about 1/3 of the formula) with continuous stirring. The uniform solution was then neutralized with triethanolamine to pH ~ 7.

Tween 20 (0.5 or 1.0 %) used to solubilize piroxicam (2 g) was added to triethanolamine/water mixture prior to incorporation into the gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

2.2.4 Piroxicam in Carbopol - 940 Gel Base Containing Brij 30 (Formulation XI and XII).

Carbopol - 940 (1.0 % W/W) was dispersed in water (about 1/3 of the formula) with continuous stirring. The uniform solution was then neutralized with triethanolamine to pH ~ 7.

Brij 30 (0.5 or 1.0 %) used to solubilize piroxicam (2 g) was added to triethanolamine/water mixture prior to incorporation into the gel base. The gel preparation was adjusted to pH ~ 8 with triethanolamine and was then adjusted to the quantity of 100 g with water.

3. pH-Measurement.

The pH measurements of all preparations were carried out by Orion pH-meter, model SA 520 A, using an electrode (Orion, Model 720). The pH-meter was standardized with standard buffer solutions. Then each preparation was measured by immersing the electrode into the formulation and the pH was read.

4. Viscosity Measurement.

The viscosities of all preparations were determined using Haake viscometer, model RC 20, RV 20 with sensor SV_2. The viscosities of gel preparations were measured with a shear rate of 40 cycles per second for four minutes at room temperature. The mean viscosity was printed out by a printer.

5. In-Vitro Diffusion Studies.

The penetration rates of piroxicam from all preparations previously described were conducted using modified Franz diffusion cells. The diffusion cell is schematically drawn in Figure 5. The inside diameter of the diffusion cells is 1.40 cm. which corresponds to the effective diffusional area of 1.54 cm^2. Polydimethylsiloxane membrane (Silastic®) was used as a synthetic model membrane and pig skin as a biological model membrane.

5.1 Preparation of Silastic® Membrane.

The 0.005" silastic® sheeting was washed out with water and rinsed with distilled water. The membrane had been equilibrated in pH 7.4 phosphate buffer for 24 hr. before it was used in diffusion studies.

5.2 Preparation of Pig Skin.

The abdominal skin of a new born pig was excised and inspected for any defects. Adhering fat and other visceral debris were carefully removed from the inner surface using a scissors. The excised skin was bathed in pH 7.4 phosphate buffer, blotted with clean cloth, wrapped in aluminum foil, and stored in a freezer. The frozen skin had been thawed before it was mounted on the diffusion apparatus.

5.3 Diffusion Studies.

A model membrane was mounted between the donor and receiver chambers of the diffusion cell using a metal clamp. The excised skin was set in place with the stratum corneum facing the donor compartment and the dermal side facing the receptor compartment. The receptor compartment was pH 7.4 phosphate buffer and was warmed to 37°C with a circulating water jacket which was connected to a constant temperature water bath. The receiving solution was kept well stirred at 300 ± 5 rpm. with a magnetic stirrer throughout the time of diffusion studies. The membrane and the receptor solution were equilibrated for 30 min. and any air bubbles that had formed

under the membrane were removed by gently rocking the cell. All diffusion studies were carried out in triplicate. After equilibration, an excess amount of piroxicam gels were applied over the membrane. Then, the donor chambers of diffusion cells were covered with paraffin films and the diffusions of piroxicam were allowed to take place.

Periodically, the receiving solution was sampled via the side arm sampling port. The entire receiving solution was removed using a syringe fitted with a piece of flexible tubing. The receiving compartment was rinsed once with pH 7.4 phosphate buffer, was replaced with fresh pH 7.4 phosphate buffer and the run was continued. Approximately five minutes were required for the sample to be removed, the cell rinsed and finally filled with fresh pH 7.4 phosphate buffer.

All receiving samples were analyzed using the HPLC technique with 0.25 mcg/ml of tenoxicam as an internal standard. Freshly prepared standard solutions were injected regularly during the analysis of each diffusion experiment. The drug concentration in the receiving side was then determined from the calibration curve. The amount permeating was calculated by multiplying the piroxicam concentration by the receiver volume. The receiver volumes varied between 5.66 and 5.96 ml.

The cumulative amount permeating was then plotted versus time. The steady-state flux was calculated by dividing the slope of the linear portion of the plot by the diffusional area.

5.4 HPLC Analysis.

The liquid chromatography was composed of a constant flow pump (Shimadzu, LC-3A), a variable wavelength UV absorption detector (Shimadzu, FLD-1), and an integrator (Shimadzu, C-R1A). Injections were made with a 200 mcl. constant-volume injector valve. The chromatograph was operated at a flow rate of 1 ml./min. and the eluent was monitored spectrophotometrically at 361 nm. The column was LyChro CART® C18 column (4 mm ID x 12.5 cm). The mobile phase was a mixture of 40% V/V methanol and 60% V/V 0.085 M monopotassium phosphate buffer (pH 5.6). Tenoxicam solution (0.25 mcg/ml) was used as an internal standard. The peak area ratio was used to determine piroxicam concentration. The run time per sample was five minutes. The retention time of tenoxicam and piroxicam were 3.2 - 3.7 min. and 4.2 - 4.8 min., respectively. The examples of chromatogram is shown in Appendix VI.

5.4.1 Preparation of Tenoxicam Solutions as Internal Standards.

Twenty-five milligrams of tenoxicam was accurately weighed in a 100 ml volumetric flask. Methanol was added and swirled until tenoxicam was completely dissolved. One ml of this solution was transferred to another 100 ml volumetric flask, and was further diluted with methanol to volume so that the final concentration of tenoxicam was 2.5 mcg/ml.

5.4.2 Stock Solution of Piroxicam for the Preparation of Standard Solutions.

Twenty-five milligrams of piroxicam was accurately weighed in a 250 ml volumetric flask and pH 7.4 phosphate buffer was used to dissolved and adjusted volume. One ml of the solution was pipetted into a 100 ml volumetric flask and again the solution was adjusted to volume with pH 7.4 phosphate buffer so that the final concentration of one mcg/ml was obtained.

5.4.3 Preparation of Standard Solutions.

0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml, respectively of the above stock solution (in 5.4.2) were pipetted and transferred to individual 10 ml volumetric flasks containing 1.0 ml of internal standard. The solutions were adjusted to volume with pH 7.4 phosphate buffer so that the final concentrations of standard solutions were 0.05, 0.10, 0.20, 0.30, 0.40, and 0.50 mcg/ml, respectively.

5.4.4 Preparation of Sample Solutions.

Five ml of receptor fluid was pipetted and transferred to 10 ml volumetric flask containing 1.0 ml of internal standard. The solution was then adjusted to volume with pH 7.4 phosphate buffer.