CHAPTER III

MATERIALS AND METHODS

Materials :

The following materials obtained from commercial source were used as received.

- Benzalkonium Chloride [Lot. No. F2002, Imperial Chemical Industries, Cheshire, Great Britain]

- Brij 35 (Polyoxyethylene (23) lauryl ether) [Lot. No. 3192649, Merck-Schuchardt, Munchen, Germany]

- Chlorhexidine Diacetate [Lot. No. 431323/4, Imperial Chemical Industries, Cheshire, Great Britain]

- Dioctyl Sodium Sulfosuccinate [Lot. No. ED361, BDH Chemicals Limeted, Poole, England]

- Ethanol AR Grade [Lot.No. 913k11532083, E.Merk, Dramstadt, Germany]

- Nifedipine USP XXI [Batch No. 313, Wilhelm Welzein & Co., Hamburg, Germany]

- Polyethylene Glycol 400 [Lot. No. PID03/1, BDH Chemicals Limeted, Poole, England]

- Pluronic F-127 (Poloxamer 407) [Batch No. 90-0513, BASF (Thailand), Bangkok, Thailand]

- Sodium Lauryl Sulfate [Lot. No. DIC32/5 , E.Merk, Dramstadt, Germany]

- Tween 80 (Polyoxyethylene (20) sorbitan monooleate) [Lot. No. TGD02, E.Merk, Dramstadt, Germany]

Apparatus :

- Analytical Balance [Sartorius 1615 MP, Germany]
- Diffusion Cell : diameter 3.5 cm. [Modified from Kesheary - Chien diffusion cell]
- Durapore 0.45 micron (HLVP 04700) [Lot. No. J+K 29045D, Millipore Corp., Massachusetts, USA.]
- Magnetic Stirrer [Magnetic - Stirrer - Hotplate, Stuart, Great Britain]
- Micropipette [Socorex, Swiss]
- pH Meter [Pye Model 232, Pye Unichem Ltd., Great Britain]
- Spectrophotometer [Spectronic 2000, Baush & Lomb, USA.]
- Thermoregular Bath [HetoFrig, Heto Birkerod, Denmark]
- Vortex Mixer [Vortex-Genie, Scientific Industrial, USA.]

Methods :

All procedures were carried out under subdued lighting conditions, glassed-wares used were amber glass or wrapped with aluminum foil, owning to the high sensitivity of nifedipine to light.

1. Preparation of nifedipine - Pluronic F-127 Gels

Every preparation consisted of the fixed concentration of nifedipine of 1%W/W.

According to the preliminary studies, it was investigated that the appropriate concentration of Pluronic F-127 Gel that exhibited suitable physical appearances as a clear, viscous and stable gel after incorporating

with nifedipine, Pluronic F-127 35%W/W gel was prepared by the cold process [BASF Waydotte, 1987b; Chen-Chow and Frank, 1981; Schmolka, 1972]: an appropriate amount of Pluronic F-127 was slowly added to 5-10°C distilled water under constant agitation, after that the dispersion was stored overnight in the refrigerator. With time, a clear, viscous solution formed. An accurately amount of nifedipine was then added to the cold solution, and the system was incubated in the gel state at 10°C until clarity was restored.

In this study, the effect of six surfactants was investigated. They were Brij 35, Tween 80, sodium lauryl sulfate, dioctyl sodium sulfosuccinate, benzalkonium chloride and chlorhexidine diacetate at 1, 3, 5%W/W. The accurately weighed amounts of the surfactants were dissolved in the water prior to preparing the Pluronic F-127 gels. For studies of the effect of surfactants to physical appearances, clarity, air bubble, rigidity were visualy observed comparing with the preparation without surfactants as control.

2. Analytical Quantitation of Nifedipine

In USP XXI, UV spectrophotometric technique was officially used for determining the content uniformity of nifedipine preparation and thin layer chromatographic technique was recommended for the assay of drug in preparation.

In this study, UV spectrophotometric technique was used for determination of the concentration of nifedipine released from the preparation according to USP XXI.

2.1 Determination of the maximum absorption wavelength of nifedipine :

Since the medium was not methanol as specified in USP XXI, the absorption wavelength was not the same as that in monograph. It is necessary to find out the maximum absorption wavelength of nifedipine in the medium of PEG 400 and ethanol cosolvent.

A solution with concentration of 0.05 mg/ml nifedipine in the medium (PEG 400 : EtOH = 1:1) was prepared for scanning to determine the maximum absorption wavelength by Spectronic 2000, The other two similar solutions were prepared to confirm the obtained wavelength.

To ensure no interference of the absorbances of surfactants or Pluronic F-127 to the observed drug absorbance, surfactants and Pluronic F-127 were tested whether they were absorbed in this obtained wavelength or not. The nifedipine solution was added into the surfactants or polymer and shaken by Vortex mixer for 15 minute. The absorbances of the supernatant solutions were observed.

2.2 The calibration curve :

The calibration curve of nifedipine was constructed spectrophotometrically for the determination of nifedipine releasing study. It was plotted between the concentration of drug against the absorbance. The series of drug in medium is in the concentration ranged of 0.01 - 0.10 mg/ml. The curve obeyed Beer's Law between 0.01 - 0.07 mg/ml. This calibration curve was used in the whole study.

3. Study of Nifedipine release from Pluronic F-127 Gel

Prior to the release study of nifedipine, all preparations, with or without surfactants, were assayed for the amount of nifedipine in preparations. The accurate 1 grams of preparations were dissolved in the 50 ml cosolvent, the absorbances of the solutions were observed.

The studies were conducted with an *in vitro* release model from the study of Gunyarat Viratyosin 1990. The release of nifedipine had been studied by an *in vitro* and *in vivo* model utilizing a membrane barrier to separate the matrix gel from a non aqueous sink. From that study, the amount of nifedipine release from Pluronic F-127 Gels *in vitro* seemed to be related to with *in vivo* study. The diffusion cell was the same as used by Sloan, K.B. and others in 1983 ; called Keshary-Chien diffusion cell .

The diffusion cell in this study consisted of a chamber (60ml) with the side arm to allow sampling of the receptor solution. The 20 grams of nifedipine preparation was accurately weighed into the donor compartment. Durapore, with the pore size 0.45 micron was clamped between the donor and the recipient compartment.

The receptor was filled with the medium solution of 1:1 Ethanol : PEG 400 cosolvent for the sink condition [Skelly et.al.1987; Kondo and Sugimoto, 1987]. A magnetic bar was stirred to provide efficient mixing. All air bubbles were removed carefully from the lower surface of membrane by tipping the cell.

Each cell was placed on a magnetic stirrer in a thermostat chamber maintained at 37°C. Samples of 1 ml were removed from the

receptor phase through the side arm at predetermined intervals; 0.5, 1, 1.5, 2 and hourly for 6 hours and every 2 hour up to 24 hours. The volume of the receptor medium withdrawn was replaced immediately with fresh warm medium of the same quantity. The amounts of nifedipine in receptor medium were calculated from the calibration curve. The results reported for each experiment were the average values from six replicate diffusion cells.

For the study of the effect of surfactants to nifedipine release from Pluronic F-127 gels. Preparations containing each surfactant at 1, 3, and 5%W/W were studied in the same manner as mentioned above. In the study, percentage and amount of nifedipine release from preparations with and without surfactants were compared.