CHAPTER III

MATERIALS AND METHODS

Materials

Clindamycin Hydrochloride Powder, USP XX, potency 836 microgram/per milligram, Batch No. 55588

Poloxamer 407 (BASF), Lot No. 62.0503

Hydroxypropyl Methylcellulose 4000, Lot No. 0485, S Tong Chemicals.

Hydroxyethyl Cellulose 4000, Lot No.4533, S. Tong Chemicals.

Bronopol(R), Lot No. 823920, S. Tong Chemicals.

Glacial Acetic Acid, K11297363, E. Merck.

Sodium Acetate, S. Tong Chemical.

Sodium Dihydrogenphosphate, A 420746, E. Merck.

Absolute Methanol, i 424607, E. Merck.

Sodium Hexane Sulfonate, Lot No. FCV 01, Tokyo Kasei Kogyo Co., LTD. Japan.

Glycerin AR Grade E. Merck, Darmstadt, Germany

Durapore(R), Diameter 47 mm, Pore size 0.45 micron, HVLP 04700, Millipore Corp. Bedford, Mass., USA.

Fluoropore(R), Diameter 47 mm, Pore size 0.5 micron, FHUP 04700, Millipore Corp. Bedford, Mass., USA.

Nylon 66, Diameter 47 mm, Pore size 0.5 micron, ES # 2024 (K29) 212, Lot. No. 562763, Altech Associate, Inc.

Phenylethyl Alcohol

Sodium Chloride, Lot.No. SEH 08/5

Equipments

Analytical Balance, H51 AR, Mettler

pH meter, Model SA 520, Orion Research Inc., USA.

High Performance Liquid Chromatography (HPLC) equipped with
Water Association 510 HPLC Pump
Water Association 848 Tunable Absorbance Detector
Water 745 B Data Module Printer
Microbondapak(R) C_{18} Column, 30 cm x 3.9 mm ID, No. 27324
Brookfield Viscometer Model DVII
Modified Franz Diffusion Cell Apparatus, Atlantic Pharmaceutical Co., Ltd.
Oven , Mammerd
Mitutoyo Micrometre
Desiccator, diameter 10 inches
Disposable needle and syringe

Methods

1. Preparation of 1% w/w Clindamycin Hydrochloride Gel.

1.1 Formulation of 1% w/w Clindamycin Hydrochloride Gel.

The gel preparations of clindamycin hydrochloride were formulated at the pH of maximum stability. Acetate buffer was chosen to maintain pH of the formulation in the range of 4.5±0.2 which is pH of human skin surface. (pH 4-6) Various synthetic gelling agents used were carbopol 940, carbopol 941, poloxamer 188, poloxamer 407, hydroxyethyl cellulose and hydroxypropyl methylcellulose. From a preliminary study, three gellants, poloxamer 407, hydroxyethyl cellulose and hydroxypropyl methylcellulose could form a proper gel in acetate buffer and were selected to further study.

The normal compositions of clindamycin hydrochloride gel formula were as follow:

	concentration (%w/w)
Clindamycin Hydrochloride	1
Glycerin	10
Bronopol(R)	0.02
Gelling agent	qs
Acetate buffer (pH 4.5) to	100

Various concentrations of gelling agents were studied. Physical appearances such as clarity, sticky, rigidity and air bubble were studied to select a proper concentration. The range of concentration of three gelling agents chosen to formulate were as follow :

a. Poloxamer 407 in the range of 15-20% w/w
b. Hydroxyethyl cellulose in the range of 1-3% w/w
c. Hydroxypropyl methylcellulose in the range of 2-4% w/w

1.2. Preparation of 1% w/w Clindamycin Hydrochloride Gel.

The proper gel bases selected from 1.1 were prepared by using various methods according to gelling agents (Appendix II) and dissolved an accurate amounts of clindamycin hydrochloride in the remained buffer with the aid of a blender. The drug solution was then added to the prepared gel base with gentle stirring. The mixtures now became clear gel.

2. Analysis of Clindamycin Hydrochloride.

There are several methods available for the determination of clindamycin in bulk drug dosage forms including a microbiological method, gas-liquid chromatography, high performance liquid chromatography. The microbiological method is time-consuming and only measures total activity. The gas-liquid chromatography method requires an extraction and a derivatization step. The high performance liquid chromatography is a rapid and accurate method. So, the high performance liquid chromatography (HPLC) using ion-pair formation, modified from Landis's method, was used in this study. (Landis, 1980)

A HPLC equipped with a variable wavelength UV absorbance detector (Water 484), chromatography pump (Water 510 HPLC Pump) and strip chart recorder (Water 745 B Data Module) was used. The HPLC operating conditions were determined as follow:

Analytical Column	Microbondapak(R) C-18 (ID 3.9 mm x 30.0 cm; partical size 10 micron)
Internal Standard	phenylethyl alcohol
Pairing-ion	sodium hexane sulfonate
UV Detector	214 nm
Flow Rate	1 ml/min
Attenuation	16
Pressure	1500 psig
Recorder Chart Speed	0.25 cm/min
Injection Volume	20 mcl

Mobile Phase

The mobile phases were composed of methanol-water (58:42), 0.1 M sodium dihydrogenphosphate and 0.005 M sodium hexane sulfonate. It was adjusted to the final pH of 6.0 with 1 M sodium hydroxide or hydrochloric acid. Mixed solution was filtered through a 0.45 micron filter and degassed by sonication and vacuum before used.

Internal Standard

Phenylethyl alcohol (1 mcg/ml) was used as an internal standard. Under the previous condition of chromatography, it had a retention time of 4.6 min.

The peak area was directly obtained from the minigrator print out and the ratio of the peak area of clindamycin hydrochloride to that of the internal standard was used to calculate the concentration of clindamycin hydrochloride in the sample.

Calibration Curve Determination.

Solution containing known amounts of standard clindamycin hydrochloride (20, 30, 40, 50, 60, 75, 100 mcg/ml) and 1 mcg/ml of internal standard in a solvent mixture of 0.01 M sodium dihydrogenphosphate in methanol-water (58:42), pH 6, which was used to dilute the analysed sample of stability and release studies, were prepared and analysed following the same

procedure as previously described. Peak area ratio of clindamycin hydrochlorede to that of the internal standard obtained versus known concentrations were fit to a straight line using the linear regression. The values of the unknown concentrations of the clindamycin hydrochloride samples were calculated from this linear equation.

3. Stability Study of Clindamycin Hydrochloride Gel.

3.1. Physical stability study.

Physical properties including viscosity, pH and physical appearances of the gel preparations were studied before and after eight Freeze-Thaw cycles (40°C, 48 hours and 8°C, 48 hours).

3.1.1 pH Measurement

All preparations were measured pH value using Orion pH-meter, model SA 520A attached to electrode, Orion Model 720. The sample was carried out by immersing the electrode into the gel preparation. The pH value was read when it appeared constant.

3.1.2 Viscosity Measurement

A Model LVTDV II Brookfield Viscometer attached to a Brookfield Helipath standard fit with a " F " size T-bar type spindle was used at a speed of 1.5 rpm, spindel T-F 96. The samples in approximate 20 g were equilibrated at room temperature (~31°C) prior to measurement. The viscosity values obtained from the reading value multiplied with 9360 then divided with speed of spindle (1.5 rpm).

3.2. Chemical Stability Study.

Approximately, each formula was weighed 500 mg to a separated tight, light resistant vial. The prepared vials were kept at:

(a) Joel-Davis condition which was performed at 40°C and 80% RH.

(b) ambient temperature which average temperature was 31°±5°C.

(c) Freeze-Thaw cycles condition.

For maintaining specified relative humidities at temperature of 40°C. A beaker of saturated salt solution of sodium chloride was placed in a closed desiccator. There must be a solid in equilibrium with a saturated solution. The desiccator was maintained at 40°C throughout the experiment by an oven. The relative humidity over this system would be 75-80% RH (Umprayn and Mendes, 1987).

All preparations were initially determined of their percent labelled amount before storing in each condition and the remaining of clindamycin hydrochloride were obtained during storage condition. The samples were collected by removing three vials of samples at appropiate time interval for about four months. Exception in the case of Freeze-Thaw cycle condition, the amounts of drug were analysed before and after this condition.

The chemical stability study was determined by the remaining of clindamycin hydrochloride during storage time and the degradation rate constant was evaluated from obtained data.

The degradation rate constant was calculated from both zero order and first order reaction. The mathematical equations of these reactions are shown as follow:

Zero order equation,

$$C = C_o - k_o t \qquad \text{.....(eq. 15)}$$

First order equation,

$$\ln(C) = \ln(C_o) - k_1 t \qquad \text{.....(eq. 16)}$$

where, C_o = the initial concentration of drug
C = the concentration of drug at time t
k_o = zero order rate constant
k_1 = first order rate constant

To determine the degradation rate constant, the clindamycin concentration and ln(concentration) were plotted against time. The zero order and first order rate constant, k_o and k_1, were obtained from the slope of linear regression, repectively.

Sample Analysis

The accurately weighed amount of 0.250 grams of sample was analysed for clindamycin hydrochloride. It was dissolved in solvent mixture, mentioned in 2, then adjusted to the final volume of 25 ml. Five ml of this solution and one ml of internal standard stock solution (10 mcg/ml) were transfered to 10-ml volumetric flask then adjusted to the final volume and mixed throughly. These solutions were analysed by HPLC technique as in 2. The drug concentrations were determined from the calibration curve. Blank gel bases were run simultaneously to check for any interferance.

4. *In Vitro* Release Study of Clindamycin Hydrochloride Gel.

In vitro release of clindamycin hydrochloride from various topical gel bases were studied utilizing modified-Franz diffusion cells and apparatus, each having the diffusional area of 1.54 cm^2. The diffusion cell is shown in Figure 3.

4.1. Preparation of Synthetic Membrane.

Various synthetic membranes were used as a model membrane. Polyvinylidene difluoride (Durapore(R)), Polytetrafluoroethylene (Fluoropore(R)), Polyhexamethylene adipamide (Nylon 66) represented hydrophilic, hydrophobic, and dialysis membrane, respectively. The approximately 1.54 cm^2 membrane was washed out with distilled water and equilibrated in receiving solution for 1 hour before using in the release studies.

In the case of Fluoropore(R) with acetate buffer system, the Fluoropore(R) must be pretreated with methanol and equilibrated in receiving solution for 1 hour.

4.2. Preparation of Receiving Solution.

Receptor phase composed of either acetate buffer or chloroform was examined. According to preventing degradation of clindamycin and being a vehicle of the formulation, acetate buffer (pH 4.5) was a suitable, aqueous

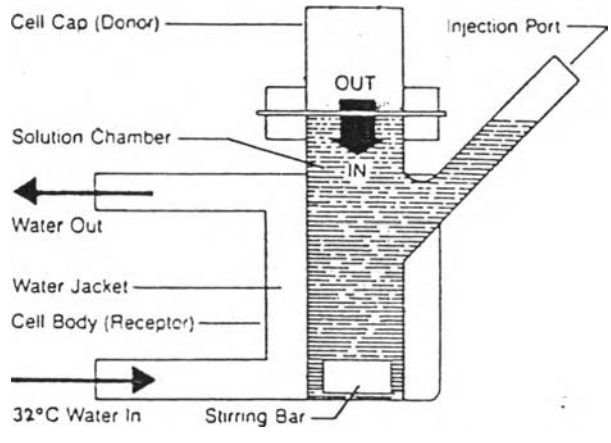

Figure 3. Schematic Diagram of Modified-Franz Diffusion Cell.

receiving solution. Nonaqueous solvent used in the experiment was chloroform which was used as a receiving solution in many researches.

4.3. *In Vitro* Release Study.

Each gel preparation approximately 2.50 gm was applied in the donor part of each modified-Franz diffusion cells and a membrane was placed over the mouth of the receiver compartment. The donor compartment was attached to the receiver chamber by a metal clamp. The donor was completely filled with the sample, and the excess of gel was removed from the surface with the edge of a spatula to obtain a smooth surface. The surface of the gel was covered with a paraffin-film. Chloroform or freshly prepared acetate buffer was filled in the receiver chamber and maintained at a constant temperature of 32°C ± 1°C (Beatrice, 1976) by means of the water jacket which was connected to water bath. The system was equilibrated for 30 minutes. Any air bubbles were removed before the sample was placed over the membrane then the release of clindamycin hydrochloride was allowed to take place. All release studies were carried out in triplicate. The solution in the receiver chamber was kept well stirred with the magnetic stirrer throughout the time of the studies.

All of samples were withdrawn at 15, 45, 75, 105,135, 165, 195, 225 and 255 minute intervals by using a syringe fitted with a flexible tubing via the side arm sampling port. The receiver chamber was rinsed once with receiving fluid and was replaced with the equal sampling volume of fresh receiving solution.

The analysis of the clindamycin hydrochloride release during the *in vitro* test was carried out by HPLC method as in 2.

As two kinds of different receiving media were used in this study, the different method to prepare the analysed samples were attained. In the case of nonaqueous receiving medium, chloroform, the sampling solution was evaporated. The dried residue was dissolved in solvent mixture, mentioned in 2, with 1 mcg/ml of phenylethyl alcohol. When acetate buffer was used, an internal standard, phenylethyl alcohol was directly added to the receiving samples. The sample concentrations were adjusted in the range of clindamycin hydrochloride standard then analysed using HPLC method as in 2. The drug concentrations in receiving solution were determined from the calibration

The cumulative amount of drug release was then plotted versus square root of time or time. The release parameter was calculated from these plots.