CHAPTER III

EXPERIMENTAL

Materials

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

Indomethacin, Batch No. 89-06-03, Vertex Chemicals Co., Hong Kong.

Absolute Ethanol, E. Merck Ltd.

Pluronic F68 and pluronic F127, BASF Ltd..

Polysorbate 80, Viddhayasom Co., Ltd..

Propylene glycol, Viddhayasom Co., Ltd..

Carbopol 940, K.H. Brothers Ltd.

Sodium hydroxide, Carlo Erba Ltd.

Elmetacin™, Batch No. HKS 15 and FKS 09, Luitpold-Werk, Germany.

Equipments

UV spectrophotometer: Spectronic 2000, The Bausch & Lomb analytical Systems Division.

Analytical Balance: Sauter Analytical Balance, August Sauter KGD-7470.

Vortex mixer: Vortex-Genie™, Scientific industries, Inc..

Shaker: Edmund Buhler TM, United Instrument Co., Ltd..

Methods

1. The Stability Indicating Assay

To study whether the UV spectrophotometer could be used for examining of indomethacin remaining in a preparation, the stability indicating assay should be performed.

The final concentration of 1 g % of indomethacin were prepared in two separate volumetric flasks. One of which used absolute ethanol as its solvent and the other used 1 N sodium hydroxide solution. Both volumetric flasks were agitated for 15 minutes. These solutions were transfered to other volumetric flasks and diluted with absolute ethanol to make about 2.5 mg/ 100 ml. The solutions were then scanned using the spectrophotometer at a wavelength range of 200-500 nm.

2. Indomethacin Solutions

2.1 Calibration Curve Solutions containing known amount of indomethacin (0.599, 1.198, 1.797, 2.396, 2.995, 3.594, 4.193 and 4.792 mg/ 100 ml) in absolute ethanol were prepared and analyzed using the UV Spectrophotometer at a wavelength of 318 nm. Absorbances obtained versus known concentrations were fitted to a straight line using the linear regression.

2.2 Solubility Estimation The solvents selected were ethanol, propylene glycol and water. A surfactant was added to each preparation as it had been expected to increase indomethacin solubility and stability. The surfactants used in this study were pluronic F68, pluronic F127, and polysorbate 80. The amount of surfactants used should exceed their critical micelle concentrations. In this study, 5 % surfactants were used. Because the strength of indomethacin solution is 1 g %, the proportion of inactive ingredients in the solvent preparations must provide the solubility of indomethacin of more than 1 g %. Ethanol content in the preparations prepared should be 60-80 % and propylene glycol content was 5-20 %.

An excess amount of indomethacin was incorporated into a screw-capped test tube containing 5 ml of the above vehicle. The test tube was swirled vigorously by the vortex mixer and placed in the shaker maintained at 25°C for 24 hours. After leaving it precipitated for 12 hours, the supernatant was pipetted and transfered into an appropriate volumetric flask and it was brought up to the final volume with absolute ethanol. The diluted solution was then analyzed using the spectrophotometer at the wavelength of 318 nm. The concentration of indomethacin was quantified utilizing the calibration curve performed previously.

2.3 Stability Study

2.3.1 Preparation of Indomethacin Solutions. Topical indomethacin solutions were prepared by dissolving indomethacin in ethanol and propylene glycol with the aid of surfactants in appropriate volumetric flasks. The final volumes were adjusted with purified water. One-ml ampules were filled with one ml of the solutions and were then kept in an autoclave at 70°C. The ampules were sampled to analyze at appropriate time intervals.

There were the total of seven topical indomethacin solutions prepared. Three preparations each of which contained 5 % pluronic F68, 5 % pluronic F127 and 5 % polysorbate 80. Another three preparations each of which contained 10 % of one of the three surfactants. Two concentrations of surfactants, 5 % and 10 %, were performed to study whether the increased amount of surfactants could increase indomethacin stability. The other one had no surfactant to examine whether the surfactants could help stabilizing the drug but indomethacin concentration must be decreased to 0.6 % because indomethacin could not dissolve completely in the solvent mixture.

From the stability data at 70°C, the more stable preparations of each surfactant were selected to study at other temperatures, i.e., at 60°C, 50°C and 40°C and ambient temperture. Drug remainings at each temperature

were analyzed using the UV spectrophometer at appropriate time intervals.

2.3.2 Stability Study of Commercial Indomethacin Solution. There is only one commercially available indomethacin solution, Elmetacin™, in Thailand. About one ml of Elmetacin™ was transfered to 1 ml ampules. These ampules were kept in autoclaves at 70°C, 60°C, 50°C, and 40°C. Some of the ampules were kept at ambient temperature. Drug remainings were then analyzed using the UV spectrophotometer at appropriate time intervals. The analysis of the commercial indomethacin solution was the same as that of the prepared indomethacin solution.

2.3.3 Sample Analysis. 0.025 ml of an indomethacin preparation was transfered to a 10 ml volumetric flask and was brought up to the final volume with absolute ethanol. The solutions were then analyzed using the UV spectrophotometer at the wavelength of 318 nm. The concentrations of indomethacin were quantified utilizing the calibration curve.

3. Indomethacin Gels

3.1 Formulation of Indomethacin Gel

Indomethacin gels were formulated by using carbopol 940 as a gelling agent. These gel preparations were composed of 20-40 % absolute ethanol as a solvent, surfactants (12-

30 % polysorbate 80, 15-30 % pluronic F127 and 20-30 % pluronic F68) as stabilizers and solubilizers, 7-10 % propylene glycol as a humectant and cosolvent, 2 % sodium hydroxide solution for adjusting the viscosity of the preparation, and purified water.

The preparations were prepared by scattering carbopol 940 in water and left them hydrated completely until they were clear and uniform. Indomethacin was dissolved in alcohol, propylene glycol and surfactant with the aid of a blender. The carbopol 940 gel bases were then added to these indomethacin mixtures with gentle stirring using a stirring rod. The mixtures now became solutions. 0.5 N. of sodium hydroxide solution was added to the gel with gentle stirring until clear gels were formed.

3.2 Stability Study The prepared indomethacin gels were packed in collapsible aluminium tubes and kept at ambient temperature. These gels were analyzed for drug remainings at appropriate time intervals.

3.2.1 Calibration Curve. The gel base was prepared by the above method but without the drug. Two grams of the gel base was dissolved with 70 % absolute ethanol in a 50 ml volumetric flask and the final volume was then adjusted. A stock solution of 1 g % indomethacin in absolute ethanol was prepared. The stock solution was transfered to eight 10-ml volumetric flasks in an amount to make the final concentration of 0.599, 1.198, 1.797,

2.396, 2.995, 3.594, 4.193 and 4.792 mg/ 100 ml. Then 1 ml solutions of the gel base were transfered to the volumetric flasks. The solutions were brought up to final volume with 70 % absolute ethanol. The solutions were analyzed using the UV spectrophotometer at the wavelength of 318 nm.

3.2.2 Sample Analysis. Two grams of indomethacin gel was weighed in a 50 ml volumetric flask. It was then dissolved and adjusted to the final volume with 70 % absolute ethanol. One ml of this solution was transfered to a 10-ml volumetric flask and adjusted to final volume with the same solvent. The solution was analyzed by the UV spectrophotometer at the wavelength of 318 nm.

4. Observations on physical changes

Changes of physical appearances of both solutions and gels such as color, odour, and clarity had been inspected visually over the time of study.