CHAPTER III

EXPERIMENTAL

Materials

Analytical grade chemicals and large volume parenteral products were obtained commercially and used without further purification. All water was deionized prior to use.

- Indomethacin, Lot No. 791001, Pharmaceutical Sciences, Ltd., Part.
- Alcohol USP, Lot No. 2438, Government Pharmaceutical Organization.
- 1,4 Dioxane, Lot No. Art 3115, E. Merck.
- Glycerine USP, Lot. No. 00126, Vidhayasom Co., Ltd.
- Polyethylene Glycol 200, Lot. No. 206520-680, Pharmaceutical Sciences, Ltd., Part.
- Polyethylene Glycol 400, Lot. No. 000127, Vidhayasom Co., Ltd.
- Propylene Glycol, Lot. No. 7107, T. Chemical Ltd., Part.
- Sorbitol Solution USP, Lot. No. 000227, Vidhayasom Co., Ltd.
- Normal Saline Solution (NSS), Lot. No. 10-094-XL-03, General Hospital Product, Co., Ltd.
- Dextrose 5% in ½ NSS, Lot. No. 62-872-XL-03, General Hospital Product, Co., Ltd.

- Dextrose 5% in Sterile Water, Lot. No. 63-923-XL-03, General Hospital Product, Co., Ltd.

- Dextrose 5% in $\frac{1}{5}$ NSS, Lot. No. 10-118-XL-05, General Hospital Product, Co., Ltd.

- Lactated Ringer's Solution, Lot. No. 11-172-XL-04, General Hospital Product, Co., Ltd.

Equipments

- UV spectrophotometer, Spectronic 2000, The Bausch & Lomb Analytical Systems Division.

- Differential Scanning Calorimeter, Model 990, Dupont.

- Digital pH meter, PBS 730, EL-Hama Instruments.

- Sauter Analytical Balance, August Sauter KG D-7470.

- Vortex, Scientific Industries, Inc.

Supplies

- Disposable micropipette tips 200 µl and 5 ml, Saha Bhasaj Chemical, Ltd.

- Disposable insulin syringes 1 ml, General Hospital Product, Co., Ltd.

- Disposable syringes 2.5, 5, 10, 50 ml, General Hospital Product, Co., Ltd.

- Disposable needles No. 18 G, 23 G, General Hospital Product, Co., Ltd.

Methods

1. Solubility Determination

1.1 Standard Curve Solutions with known amounts of indomethacin (1, 2, 2.4, 3, 4, 5, 6, 10 µg/ml) in water were prepared and analyzed using a UV spectrophotometer at 318 nm (3,6). Absorbances obtained versus known concentrations were fitted to a straight line using linear regression (25).

1.2 Mixed Solvents Preparations The solvents selected to form a binary solvent with water were alcohol, glycerine, propylene glycol, polyethylene glycol 200, 400, sorbitol solution and 1,4 dioxane. The concentrations of these solvents and water were varied from 10 percent to 90 percent v/v (Appendix). About 50 ml of individual mixed solvents was prepared. Exactly 20 ml was employed as solvent for indomethacin. The remainder was determined for its density utilizing a pycnometer.

1.3 Drug Incorporation One gram of indomethacin was incorporated into parafilm-capped test tubes containing 20 ml of each mixed solvents. The test tubes were vortexed well and placed in a water bath maintained at about 50℃ for 3 minutes. Finally, the tubes containing indomethacin in mixed solvents were stored at room temperature (30 ± 1℃) for 7 days. Preliminary studies indicated that this period was sufficient to ensure saturation (28). After equilibrium was attained as seen by no further crystal growth, the solutions were filtered using filtered papers to remove an excess insoluble drug. The clear solutions obtained were determined for indomethacin concentrations, solutions density and stored at room temperature for at least 2 months for stability study.

1.4 Sample Analysis Aliquots of indomethacin solutions were transferred into appropriate volumetric flask and brought up to the final volume with water. Solutions were then analyzed using a spectrophotometer at 318 nm (3,6). The concentrations of indomethacin were quantified utilizing a standard curve.

1.5 Molar Heat of Fusion Determination Due to the ΔHf value of indomethacin is not available in literatures. The value used for calculation is determined using a differential scanning calorimeter. Indium was used as a standard. The total heat utilized for melting indium and indomethacin were automatically recorded. The ΔHf value of indomethacin was calculated following the method presented in Chapter II.

However, the ΔHf value is simply determined in a laboratory as described in Chapter II. An excess amount of indomethacin was incoporated into 5 parafilm-capped test tubes containing 20 ml of water each. Each tube was placed in a shaking water bath maintained at 30°, 40°, 50°, 60° and 70°C, respectively, for 8 hours. Solubilities of indomethacin in water at these temperatures were quantified employing spectrophotometer and standard curve. The ln mole fraction solubilities of indomethacin in water versus the reciprocal temperatures, ^{o}K, were fitted into a straight line using linear regression (25). The ΔHf value was then calculated from slope of the line.

1.6 Calculation of Solubility Parameters and Molar Volumes for Solute and Some Pure Solvents

The δ_2, V_2 of indomethacin; δ_1, V_1 of sorbitol solution, polyethylene glycol 200 and polyethylene glycol 400 were calculated using group contribution method (23). All other values were taken from previously published data.

1.7 Calculation of Solubility Parameter, Volume Fraction and Mean Molar Volume of Mixed Solvents

All these values were obtained using Eqs. 19, 20 and 21, respectively.

1.8 Calculations of Ideal Solubility, Activity Coefficients, and Observed values of solute-solvent interaction energy

The method begins with a calculation of the ideal solubility, x_2^i, of indomethacin. This is obtained using Eq. 3. The logarithm of indomethacin activity coefficient is calculated using Eqs.12,13. Log γ_V and log γ_R are obtained using Eqs. 14 and 15, respectively. Observed values of W, the solute-solvent interaction energy ,are calculated using Eq. 17.

2. Compatibility and Stability Study Selected indomethacin solution was prepared using an appropriate mixed solvents for compatibility and stability test. This solution suitably provided concentrations and viscosity for use as pharmaceutical dosage forms. Systems involved with this experiment were :

2.1 Compatibility and Stability in Buffer Solutions

Sorensen's phosphate buffer solutions at various pHs (5.9, 6.5, 7.0, 7.4 and 8.0) were prepared (Appendix). Exactly 10 ml of each pH buffer solution was transfered into numbers of erlenmyer flasks. The exact amount of selected indomethacin solution was incrementally added starting with 1 ml at the first flask and so on. After mixing well, physical incompatibilities such as precipitation, discoloration or separation in each mixed solution were observed. Only clear solutions were analyzed using spectrophotometer for indomethacin contents and stored at room temperature for at least 72 hours (28). Mixed solutions were then reobserved and reanalyzed. The results obtained before and after storing were compared.

2.2 Compatibility and Stability in Large Volume Parenteral (LVP) Solutions All commonly used LVP were obtained commercially. They are; Normal Saline Solution (NSS), Dextrose 5% in $\frac{1}{2}$ and $\frac{1}{5}$ NSS, Dextrose 5% in Sterile Water and Lactated Ringer's Solution. The procedure used in this study is similar to that of 9.1 except the exact volume of LVP is 50 ml and the exact volume of indomethacin solution incrementally added was started with 0.25 ml.