CHAPTER III

MATERIALS AND METHODS

Materials

Analytical grade chemicals were obtained commercially and used without further purifications. All water was distilled prior to use.

- Phenobarbital B.P., Lot No. 27358, BDH Chemical Ltd.
- Sulfadiazine B.P., Lot No. UM 11,
  Srichand United Dispensary Ltd., Part.
- Diethyl ether, Lot No. Art 921, E. Merck.
- Ethyl acetate, Lot No. 067339, Riedel-De Haenag, Germany.
- Chloroform, Lot No. Art 2445, E. Merck.
- 1, 4 - Dioxane, Lot No. Art 3115, E. Merck.
- Tert-Butyl alcohol, Lot No. 6249370, BDH Chemical Ltd.
- Amyl alcohol, Lot No. BC 01063, May & Baker.
- Sec-Butyl alcohol, Lot No. BX 01159, May & Baker.
- Isobutyl alcohol, Lot No. B 8904, May & Baker.
- N-Butyl alcohol, Lot No. EB 00853, May & Baker.
- Isopropyl alcohol, Lot No. Art 9634, E. Merck.
- N-Propyl alcohol, Lot No. 2265610, BDH Chemical Ltd.
- Acetonitrile, Lot No. Art 30, E. Merck.
- Ethylene glycol monomethyl ether, Lot No. CVK, Mallinckrodt Inc.
- Ethyl alcohol, Absolute, Lot No. 8765, T. Chemical Ltd., Part.
- Methyl alcohol B.P., Lot No. 750617, Vidhyasom Co., Ltd.
- Propylene glycol USP, Lot No. 2876, T. Chemical Ltd., Part.
- Ethylene glycol, Lot No. KMPG, Mallinckrodt Inc.
- Glycerin USP, Lot No. 00126, Vidhyasom Co., Ltd.

Equipments

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

- Differential Scanning Colorimeter, Model 990, Dupont.

- Julabo constant temperature shaker bath, Model SW 1-V Julabo Juchhiem Labortechnik KG, West Germany.

- Mettler Analytical Balance, H-51 AR

- Pycnometer

- Vortex, Scientific Industries, Inc.

Methods

1. Solubility Determination

1.1 Preliminary Test Solutions with known amounts of phenobarbital and sulfadiazine in various individual solvents were prepared and analyzed using a UV spectrophotometer at 240 nm. and 254 nm. (31, 32), respectively, to obtain the best absorbance within the range of 0.2 - 0.7 (31).

1.2 Standard Curve Solutions with known amounts of phenobarbital and sulfadiazine in various individual solvents from 1.1 were prepared and analyzed using a UV spectrophotometer at 240 nm. and 254 nm., respectively. Absorbances obtained versus known concentrations were fitted to a straight line using linear regression (33).

1.3 Preparation of Test Solutions Excessive amounts of phenobarbital and sulfadiazine were added into 15 ml of each individual solvent in screw-capped test tubes. After mixing well using a vortex mixer, the test tubes were placed in a constant temperature shaker-bath maintained at 30 ± 1°C. The tubes containing each drug

in individual solvent were shaken at 100 cycles/min for 48 hours. Preliminary studies showed that this time period was sufficient to assure saturation at 30°C (18). After equilibrium was attained, the tubes were removed to analyze for drug concentrations. The saturated solutions were filtered using filtered papers to separate an excess insoluble drugs in order to obtain clear solutions. The resulted clear solutions were determined triplicately for phenobarbital and sulfadiazine concentrations in each solvent.

1.4 Sample Analysis Aliquots of phenobarbital and sulfadiazine solutions were transferred into appropriate volumetric flask and brought up to the final volume with each solvent used. Solutions were then analyzed using a UV spectrophotometer at 240 nm and 254 nm (31, 32). The concentrations of phenobarbital and sulfadiazine were quantified utilizing standard curves.

2. Density Determination

The clear solutions obtained from 1.3 were determined triplicately for solutions density using a glass pycnometer.

3. Molar Heat of Fusion Determination

Since the ΔHf value of phenobarbital is not available in literature. The value used for calculation is determined using a differential scanning colorimeter(8,34). Indium was used as a standard. The total heat utilized for melting indium and phenobarbital was automatically recorded. The ΔHf value of phenobarbital was calculated employing an equation (4, 35):

$$\Delta Hf\ (sample) = \left[\frac{\text{Sensitivity for sample} \times \Delta Hf\ (Std) \times \text{Std. wt.}}{\text{Sensitivity for std.} \times \text{sample wt.} \times \text{Std. MW}} \times \frac{\text{Sample peak area} \times \text{Sample MW.}}{\text{Std. peak area}} \right] \quad \text{(Eq.23)}$$

4. Calculation of Solubility in Mole Fraction

The observed mole fraction solubilities ($X_{2\ obs}$) of phenobarital and sulfadiazine in each individual solvent were calculated using the following equations (1, 27, 35):

Grams of solution = volume x density (Eq.24)

therefore; Grams of solution/L = 1000 × density (Eq.25)

and ; Grams of solute/L = concentration (μg/ml)/$_{1000}$ (Eq.26)

Grams of solvent/L = Grams of solution/L - Grams of solute/L (Eq.27)

moles of solvent (n_1) = $\frac{\text{Grams. of solvent}}{\text{MW. of solvent}}$ (Eq.28)

moles of solute (n_2) = $\frac{\text{Grams of solute}}{\text{MW. of solute}}$ (Eq.29)

$$X_{2\ obs} = \frac{n_2}{n_1 + n_2}$$

5. Calculation of Observed Values of ϕ_1 and A

All these values were obtained using Eqs. 12 and 11, respectively.

6. Calculation of the Logarithm of Ideal Solubility ($\log X_2^i$)

All these values were obtained using Eq. 3

7. Calculation of Predicted Mole Fraction Solubilities of Phenobarbital and Sulfadiazine in Various Individual Solvents.

7.1 Using Method of the Regular Solution Theory (Scatchard-Hildebrand Equation)

The predicted value of X_2 was achieved by employing Eqs. 12, 11, and 14, respectively, begining with a value of 1.0 for ϕ_1 and iterating until X_2 or ϕ_1 no longer changed by more than some desired small value, say 1×10^{-5}.

7.2 Using Method of the Extended Hildebrand Solubility Approach (EHS)

The observed values of W of phenobarbital and sulfadiazine for each solvent were calculated using Eq. 15 from knowing other terms as reported earlier.

The observed values of W are regressed versus the total solubility parameter of each solvent (δ_1) in a polynomial expression as Eq. 17 using the computer program regression analysis. Then, back calculating W and substituting into Eq. 16.

Finally, the predicted value of X_2 was achieved by employing Eqs. 12 , 11, and 16, respectively, begining with a value of 1.0 for ϕ_1 and iterating until X_2 or ϕ_1 no longer changed by more than some desired small value, say 1×10^{-5}

7.3 Using method of the Extended Hansen Solubility Approach

The observed values of $\log \left[X_2^i/X_2\right]$ or $\log \alpha_2$ of phenobarbital and sulfadiazine for each individual solvent were

calculated using Eq. 6 from knowing the observed values of X_2, ϕ_1, A and log X_2^i as reported earlier.

Then the observed values of log $\left[X_2^i/X_2\right]$ or log α_2 are regressed versus a multiple regression expression against A $(\delta_{1D}-\delta_{2D})^2$, $A(\delta_{1P}-\delta_{2P})^2$, and $A(\delta_{1H}-\delta_{2H})^2$ using the computer program regression analysis from knowing partial solubility parameters of solutes and solvents obtained from the literature.

Finally, the predicted value of X_2 of each drug for each solvent was achieved by employing Eqs. 12, 11, and 22, respectively, begining with a value of 1.0 for ϕ_1 and iterating until X_2 or ϕ_1 no longer changed by more than some desired small value, say 1×10^{-5}.

8. Comparison of the predicted solubilities ($X_{2\,calc}$) VS the observed solubilities ($X_{2\,obs}$)

The predicted solubilities ($X_{2\,calc}$) of phenobarbital and sulfadiazine for each solvent obtained from three different methods mentioned above were compared among each others and those of the observed solubilities ($X_{2\,obs}$) of each drug in each individual solvent using residual method.