CHAPTER V

DISCUSSION

1. Characterization of pralidoxime chloride starting material

The experiment was done to obtain solid state reference data when characterizing of pralidoxime chloride. These data were used to compare with the freeze dried finish product when freeze drying experiment is done. It is important to confirm the similarities and the differences between freeze dried product and the starting material powder.

Differential scanning calorimetry (DSC) thermogram showing the thermal behavior of pralidoxime chloride starting material shows the melting point with an onset temperature of about 220.5°C and the peak temperature at 224°C (Figure 11). According to Merck Index (1), the melting point of pralidoxime chloride is reported to be between 222-225°C. The thermogram represents the melting endotherm first, and then shows the recrystallization exotherm, then the second melting endotherm with subsequent thermal degradation. DSC thermogram indicates that pralidoxime chloride melts first and follows with the recrystallization to the new form. After this form melts, the degradation occurs immediately after melting. The suggestion from this DSC study is that pralidoxime chloride contains two forms that change after melting shown by the thermogram describes above.

XRPD shows the highly crystalline pralidoxime chloride starting material powder as indicated by the strong and sharp peak intensities. The XRPD result is in good agreement with the SEM photomicrograph that it exhibit smooth and homogeneous surface with small crystals scattering on the surface. The size of the crystal is possible to conclude that the particle size may be highly dispersed

Infrared spectrum was done to obtain the structural aspects. The principle peaks of IR spectrum of pralidoxime chloride starting material are aromatic compound at wave number around 1500 cm^{-1} which are possibly phenoxy or amino substituents and an aromatic tertiary amine at wave number 2700 cm^{-1} (Figure 12) according to the structure of pralidoxime chloride. When compared with the standard infrared spectrum obtain from The Aldrich Library of Infrared Spectrum (figure13), the principle peak groups are located at the same position.

2. Thermal analysis of pralidoxime chloride frozen solution

Low temperature thermal analysis was investigated and was divided into two experiments, frozen behavior with and without thermal treatment (annealing).

2.1 Frozen behavior without thermal treatment

DSC was used to detect a change in thermal behavior of the frozen solution. The scan rates for cooling and heating were 0.5°C/min because the high heating rate may cause loss of resolution of the closely spaced endotherms in the eutectic melting region. During freezing, DSC thermogram reveals the exothermic peak at -20°C (data not shown). This exothermic event usually calls supercooling. There is no endothermic base-line shift indicative of the occurrence of glass transition of amorphous material. DSC thermogram (Figure 14) shows three prominant endothermic peaks indicating that pralidoxime chloride formed eutectic mixtures with water. The first and second endothermic peaks (peak A and B from Figure 14) represent the melting of the eutectic mixtures consisting of finely divided crystals of ice and pralidoxime chloride in two different frozen configurations. The temperatures at which this occurs are called the eutectic melting temperatures. When focused on the endothermic melting peaks, the first endothermic peak at -12°C (peak A) is bigger than the second endothermic peak at -10.3°C (peak B). These endothermic peaks represent the two eutectic form combine in the frozen solution. Thus, it was assumed that the separation and isolation of the two forms may be done by thermal treatment or annealing. The annealing at appropriate temperature around eutectic melting point could initiate the new more stable form of pralidoxime chloride to finally reach nearly 100% purity.

From the study of glycine/water frozen solution characterization by Chongprasert et. al. referred that different low temperature characterization of glycine frozen solution can initiate different polymorph by manipulating the freezing and freeze drying conditions. Thus, the low temperature thermal analysis of pralidoxime chloride solution was set to better understand the low temperature behavior of frozen solution. The DSC thermograms of frozen glycine solution were performed. Different forms of glycine that came from the different annealing temperatures at controlled temperature in the melting region (Figure 41). The manipulation of the thermal history of a quench cooled glycine solution that allows three of four endotherms prior to ice melting to be identified.

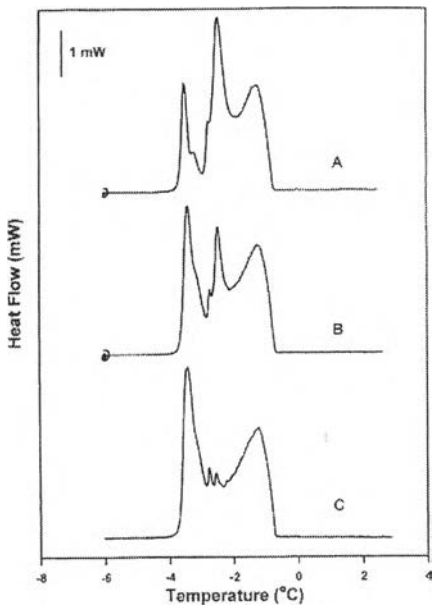

Figure 41 The manipulation of the thermal history of glycine at different annealing conditions (12)

The cooling and heating rate variation (Figures 15-22) has no influence to the low temperature thermal behavior of pralidoxime chloride frozen solution. The thermograms show that two eutectic melting endotherms obtained are not different from the endotherms of pralidoxime chloride starting material. The experiment was established to find the best resolution for DSC scans and a result able to select the suitable heating and cooling rates for future experiments. The cooling and heating rates selected from this experiment were 20°C/min and 1°C/min, respectively (Figure 15D).

2.2 Frozen thermal behavior with thermal treatment.

The experiment was set in two conditions following the previous, annealing before and after reaching the predetermined freezing temperature. The annealing temperatures were set around eutectic temperature at -12, -15 and -17°C.

2.2.1 Annealing before the predetermined freezing temperature

The expected thermal behavior from this condition is the thermal behavior in the same pattern of starting material. From the DSC result (Figure 23) revealed the same pattern with starting material. The molecules arrange in the same structure as the starting material. Annealing did not affect this condition. The DSC thermograms support the expectation phenomenon. The reasons are describe below.

- Pralidoxime chloride has the degree of supercooling at -20°C. The solution is not frozen at the annealing temperatures used. The crystal structure of frozen solution did not form. Thus the rearrangement of the structure did not occur. The structure is still present at the same form with starting material.
- Annealing time for one hour is insufficient to allow the crystal structure rearrange the molecule into new form.

2.2.2 Annealing after the predetermined freezing temperature

The hypothesis for this experiment is described below.

- At annealing temperature -12°C, pralidoxime chloride should change from the first eutectic form (eutectic melting at -12°C) to deposit onto the second eutectic form (eutectic melting at -10.3°C).
- At annealing temperature -15 and -17°C, the different thermal behavior should not occur because the crystal structure was not allowed to move or rearrange at temperatures lower than eutectic melting temperature.

DSC thermograms (Figure 24) show that the results are different from the expectation. There is no thermal behavior change at any annealing temperatures. The explanation is that the annealing time allows is insufficient to change the molecular structure to the new form. This result might be the systematic errors of the experiment or the substance specific of pralidoxime chloride. Thus, the result does not support the expectation and different from the result of glycine/water system.

3. Freeze drying experiment and characterization of freeze dried pralidoxime chloride

Freeze drying experiments were performed to confirm the conclusions of the low temperature thermal analysis to determine whether the crystal form can be controlled by thermal treatment at the appropriate conditions during freeze drying process. Thus, the experiments were set to follow the DSC experiments.

3.1 Annealing before the predetermined freezing temperature

The results from DSC thermogram of freeze dried products follow the hypothesis suggested that the DSC thermogram are not different from DSC thermogram of starting material.

- At annealing temperatures of -12 and -15°C (Figures 35), DSC thermograms show different patterns when compared to thermograms at

different annealing conditions. The suggestion is describe that the system may consist of more than one form. The thermograms represent recrystallization occurred with concomitance degradation.

- At annealing temperature -17°C (Figure 35), thermogram shows the broad endothermic peak with the immediately recrystallization exotherm and follows by the melting with degradation of products.

From the results, DSC thermograms of freeze dried products (Figures 35) show the broad endothermic melting peak. The expected phenomenon is these products contain the loosely network structure which did not arrange in the most ordered conformations. Thus, the structure is less stable and the broad melting endotherm show the onset of approximately 215°C and end at 230°C. At every nnealing temperature used, the drug did not freeze because the super cooling occurs at temperature of -20°C below the annealing temperature. The system was still cool solution. Annealing at this condition should not induce any expected phase transformation by the reasons given above.

Refer to the XRPD (Figure 27), the x-ray diffractogram XRPD were identically between the product of this condition and the starting material. The x-ray patterns show crystallinity but less than the starting material. Freeze drying process usually generate the freeze dried product with low crystallinity which the molecular structure did not have sufficient time for arrangement.

3.2 Annealing after predetermine freezing temperature

The freeze dried products were initially assumed that new solid form could be generated when annealed at various conditions. The system was already frozen and ready to melt and reconfigure at the annealing temperatures situated around eutectic temperature for phase transformation.

DSC thermograms of freeze dried products (Figure 36) show the onset of the very sharp melting endotherm at approximately 225°C that is higher than the melting point of starting material. The suggestion is that the more stable form occurred by using this annealing condition. The system rearranges the structure into more ordered structure than the condition of annealing before the predetermined freezing temperature, resulting in the higher and sharper melting point. The conclusion is that all molecules obtained by this condition melt at the same time and temperature.

The XRPD patterns of freeze dried product by this method show less crystalline than the XRPD of starting material and the product of annealing before the predetermined freezing temperature condition. The results from XRPD are not consistent with DSC results. This result implies that it deserves further investigation in the future. This is due to the fact that the solution was frozen very rapidly from room temperature to -40^{o}C. It was assumed that this rapid cooling caused very poor arrangement of the drug. Drug molecules or group of molecules are trapped in isolated sites divided by the solid structure of ice. It is so finely divided that even after annealing and drying, it still show patterns similar to that of amorphous material (Figure 28) because the ice lattice did not melt. When water was removed, the structure shows small flakes as very fine powder-like material which XRPD can not be determined as a regularly spaced lattice as seen in crystalline structure. It is known for a fact that particle size may have a considerable effect on the resulting XRPD patterns. The products obtained are loosely packed.

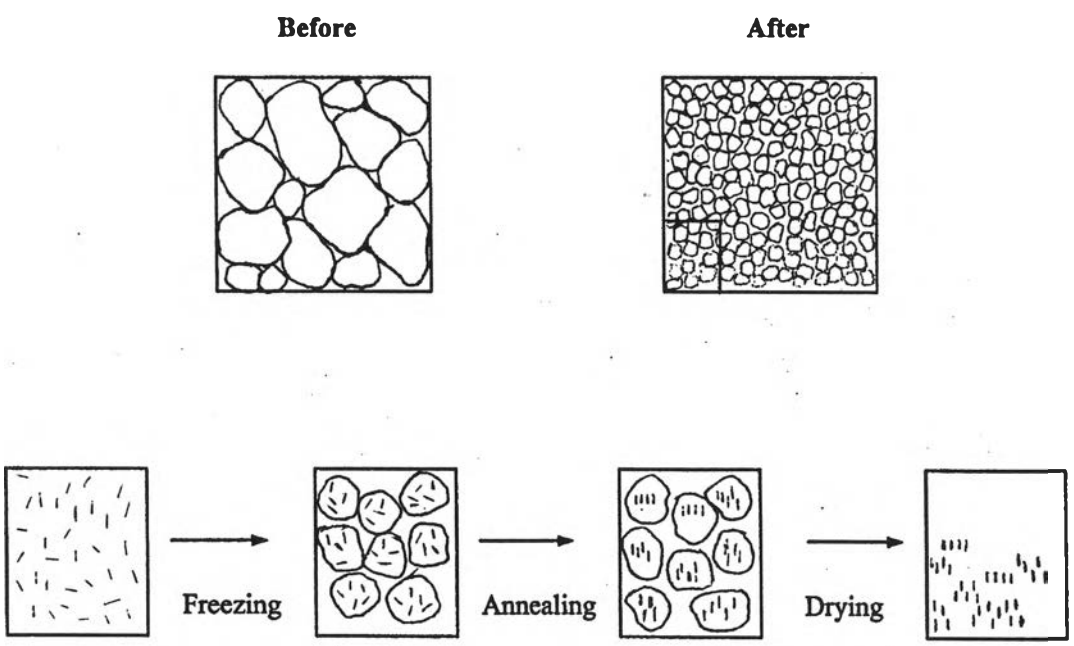

Figure 42 Phase transformation of freeze dried pralidoxime chloride at annealing after the predetermined freezing temperature condition

4. Stability of freeze dried pralidoxime chloride

The solid state stability of freeze dried pralidoxime chloride was observed under the humid condition and dry condition. In this experiment, the freeze dried product used was the one that annealed after the predetermined freezing temperature because of its high amorphous content of this product which made it very unstable.

At dry condition (40°C, 0% RH), all of freeze dried pralidoxime chloride cakes were found to only slightly collapse after five days experiment (Figure 37). The x-ray powder diffractogram revealed that the crystallinity and the form of freeze dried products were similar for up to five days (Figure 39). There were no significant changes in the x-ray powder diffractogram of all the products. There were no water sorption occurred in the system because of the condition that was set. This result indicated that the temperature of the experiment at 40°C could not alter the solid structure of the freeze dried pralidoxime chloride and they still exist as partially crystalline material.

After exposing to a humid condition (40°C, 70% RH), the product was found to collapsed after the first day of the experiment (Figure 38). The product looked moist. The x-ray powder diffractogram represent that the crystallinity of freeze dried products increased as time of experiment increased (Figure 40). On the fifth day, the product was mostly crystalline.

The suggestion of this phenomenon is that the products uptake the moisture and change physicochemical characteristic from low crystallinity to higher crystallinity by underwent moisture mediated molecular loosening and rearrangement to a more stable structure. Moisture induces the phase transformation. No change was observed when kept in dry atmosphere at elevated temperature. This suggestion is proved from the result of the experiment of dry condition product above. From the stability experiment, an important suggestion about the storage condition of pralidoxime chloride freeze dried product was recognized. The product should be kept in an air tight container to avoid moisture that can change it to a more crystalline material, which will eventually affect the reconstitution behavior of the freeze dried product.

5. Practical Implication

This investigation is useful on the manufacturing perspective. Annealing process should only be done when the solution was already frozen. The annealing before the predetermined freezing temperature condition obtained the same structure as with the starting material. The

manufacturer should manipulate the conditions and the industrial process during the change from the solution form to freeze dried product with the same chemical properties but better physicochemical properties.

DSC and XRPD are useful tool to gain a better understanding of the complex behavior of a simple system such as the system of pralidoxime chloride in water, required expertise in the area. Thus manipulation of the thermal behavior of pralidoxime chloride solution using DSC during preformulation can improve the understanding of the overall behavior of the product during freeze drying and use this finding to differentiate the different form of drug to select the most desired form for optimal efficacy.