CHAPTER III

EXPERIMENTAL

1. Drug - Pralidoxime Chloride (Lot. No. 60H0445, Sigma Chemical Co., USA)
2. Chemical - Deionized water
   - Double distilled water
3. Equipments - Analytical balance (Model BA210S Basic, Sartorius, Germany)
   - Analytical balance (Model AB204, Mettler-ToledoGmbH, Analytical, Switzerland)
   - Differential scanning calorimeter (Model DSC822e, Mettler, Switzerland)
   - Fourier transform infrared spectrometer (Model FT-IR 1760X, Perkin Elmer, USA)
   - Scanning electron microscope (Model JSM-5410LV, JEOL, Tokyo, Japan)
   - X-ray powder diffractometer (Model X'PERT system PW 1827/91 Instrument, Philips, Netherland)
   - Freeze dryer (Dura-Stop^{TM} Digital Programmer Stoppering Tray Dryer and Dura-Dry^{TM} MP Microprocessor Control, FTS System^{TM}, Stone Ridge NewYork, USA)
   - Controlled temperature oven (Model BD/ED/FD with R3-Controller, WTB Binder)

Methods

1. Characterization of pralidoxime chloride starting material

1.1 Morphology study

The study was to observe the habits of differing crystal structures. The morphology of pralidoxime chloride starting material was characterized using the scanning electron microscope.

Sample preparation for imaging was by mounting on a sample mount using double-sided tapes. Sample required vapor deposition of a thin metal coating using a sputter coater. Gold metal was used for coating. The coating reduced charge buildup on the specimen under the electron beam, resulting in clear, stable images.

1.2 X-ray powder diffraction analysis

The x-ray powder diffractometer was used to obtain fingerprint x-ray powder diffractogram of pralidoxime chloride starting material. Pralidoxime chloride starting material was ground lightly and placed in a quartz sample holder for which it was scanned from $5^{\circ} - 40^{\circ}$ 2θ at a rate of 0.02 step/s.

1.3 Thermal analysis

Differential scanning calorimetry (DSC) was used to characterize the thermal behavior of pralidoxime chloride starting material. About 5-10 mg of pralidoxime chloride starting material was placed in an aluminium pan and hermetically sealed with an aluminum cover. Another empty aluminum pan with cover was used as a reference. Nitrogen was used to purged the sample chamber. Temperature was calibrated using indium. Thermogram was recorded during heating at a scanning rate of 10°C/min from 25°C to 300°C.

1.4 Infrared Spectrophotometry

The infrared absorption spectra appropriate for the characterization of polymorphs and solvates was best obtained using Fourier transform infrared spectroscopy–FTIR. Sample was prepared with KCl pellets without grinding.

2. Thermal analysis of pralidoxime chloride frozen solution

Freeze-drying condition was simulated using the DSC as a tool. Aqueous sample solution of the drug will be exposed to different thermal events such as varying cooling rates, heating rates and annealing temperatures. Low – temperature thermal analysis was carried out using a heat flux DSC equipped with electrical cooling unit. Temperature calibration was done using the melting point of indium (m.p. 156.60°C).

2.1 Frozen behavior without thermal treatment

2.1.1 Preliminary freezing study

Approximately10 µl of 5% w/v pralidoxime chloride solution was frozen at cooling rate of 0.5°C/min from 25°C to -40°C and heated up at heating rate 0.5°C/min to 25°C. Thermograms were recorded during the cooling and heating cycle. The cooling and heating rate was set at 0.5°C/min for identifying the events at low temperature thermal characteristics.

2.1.2 Variation of cooling and heating rates

Approximately10 µl of 5% pralidoxime chloride solution was hermetically sealed in an aluminum pan. An empty aluminum pan with lid was used as a reference. Samples were cooled from room temperature (25°C) to final freezing temperature (-40°C) and heated from freezing temperature to 10°C at different cooling and heating rates cycles. Thermograms were recorded during the cooling and heating cycle at various cooling and heating rates. The variation of 2 factors, cooling and heating rate, are demonstrated in Table 1.

Table 1 The variation of cooling and heating rate for the DSC method.

Sample	Cooling rate (°C/min.)	Heating rate (°C/min.)
1	1	1
2	1	5
3	1	10
4	1	20
5	5	1
6	5	5
7	5	10
8	5	20
9	10	1
10	10	5
11	10	10
12	10	20
13	20	1
14	20	5
15	20	10
16	20	20

2.2 Frozen behavior with thermal treatment

2.2.1 Annealing before the predetermined freezing temperature

Approximately 10 µl of 5% pralidoxime chloride solution was hermetically sealed in an aluminum pan. An empty aluminum pan and lid was used as a reference. Samples were cooled

from room temperature (25°C) at 20°C/min to annealing temperature and annealed for 1 hour and then cooled to final freezing temperature (-40°C) and heated from final freezing temperature at 1°C/min to 10°C. Thermograms were recorded during the cooling and heating cycle. For this experiment, samples were annealed around the eutectic melting temperature, which was determined prior to this experiment, before reaching final freezing temperature. From the result, choose the best resolution of DSC thermogram to represent the appropriate cooling and heating rate for varying annealing temperature measurements. The variation of annealing temperatures was -12, -15 and -17°C.

2.2.2 Annealing after the predetermined freezing temperature

Approximately 10 µl of 5% pralidoxime chloride solution was hermetically sealed in an aluminum pan. An empty aluminum pan and lid was used as a reference. Samples were cooled from room temperature (25°C) to final freezing temperature (-40°C) at cooling rate 20°C/min and heated from final freezing temperature to the annealing temperature for 1 hr and heated up to 10°C at heating rate 1°C/min. Thermograms were recorded during the cooling and heating cycle. For this experiment, samples were annealed at temperature after final freezing temperature. From the result, choose the best resolution of DSC thermogram to represent the cooling and heating rate for varying annealing temperature measurements. The variation of annealing temperature were -12, -15 and -17°C.

3. Freeze drying experiment

From the simulation of freezing conditions, desired temperature used during freezing cycle to produce desired solid phase are well defined. The appropriate sample solution will undergo freezing cycle using temperature reminiscent to that obtained from DSC to produce the desired solid structure for future benefits.

Freeze drying experiment was done using a laboratory freeze-dryer. Approximately 2 ml of 5% w/v pralidoxime chloride solution was transferred to 5 ml glass vials. Rubber lyostoppers were used. Freezing was done by cooling to designated temperatures in the freeze dryer. Freeze drying conditions were set at the 2 conditions following DSC simulation in experiment 2.2. Samples were held in the freeze dryer at the annealing temperatures for 6 hours for annealing. Primary drying was set by following programmed temperature to step up the

temperature gradually until the temperature reach $0^{\circ}C$ on the process time of 12 hours. The programmed temperature was set from these details. First, the temperature was increased $10^{\circ}C$ in each two hours after the pressure was decreased below 100 mTorr. The temperare was increased until it reached $0^{\circ}C$ then hold sample at this temperature until 12 hours of primary drying process. Secondary was followed by increased the temperature gradually like primary drying until the temperature reach $25^{\circ}C$ for 12 hours from the data reported by Chongprasert et al. Product temperature was measured by 32-gauge type T thermocouples. Chamber pressure was controlled below 100 mTorr throughout the drying cycle. The samples were stoppered under vacuum.

3.1 Annealing before the predetermined freezing temperature

Samples were cooled by loading onto precooled shelves to the desired annealing temperature (results obtained from DSC simulation in experiments 2.2). Samples were held in freeze dryer at the annealing temperature for 6 hrs. Annealing was carried out at various temperatures. After annealing, samples were frozen to temperature $-40^{\circ}C$ for complete solidification for 1 hr, then heated up to the primary drying temperature, but below the eutectic melting temperature. Primary drying was set for 12 hrs at the process below 100mTorr and secondary drying was set for 12 hrs at temperature $25^{\circ}C$. Products were stoppered under vaccuum after drying cycle was done.

3.2 Annealing after the predetermined freezing temperature

Samples were frozen by loading onto precooled shelves at frozen temperature, $-40^{\circ}C$. Samples were held at this temperature about 1 hr for complete solidification. After freezing, sample were heated up to the annealing temperatures, at -12, -15 and $-17^{\circ}C$, and held in this temperature for 6 hrs for annealing. Samples were heated up after annealing to the primary drying temperature and dried for 12 hrs. On the last process, secondary drying was done at $25^{\circ}C$ for 12 hours.

4. Characterization of freeze dried pralidoxime chloride

The freeze dried pralidoxime chloride will be subjected to the following analytical procedures.

4.1 X-Ray Powder Diffractometry (XRPD)

The x-ray powder diffractometer was used to obtain x-ray powder diffractograms of freeze dried pralidoxime chloride by using a Philips X'PERT XRPD. The sample was ground and placed in a quartz holder and scanned from $5^{\circ} - 40^{\circ}$ 2θ at a rate of 0.02 step/s.

4.2 Scanning Electron Microscopy (SEM)

The morphology of freeze dried pralidoxime chloride is characterized to observe the habits of differing structure using scanning electron microscopy.

4.3 Thermal analysis study

Differential Scanning Calorimetry will be used to characterize the thermal behavior of freeze dried pralidoxime chloride. In DSC method, the sample and reference materials are maintained at the same temperature and measure the heat flow required to keep the equality in temperature that represent in DSC thermogram. About 5-10 mg of freeze dried pralidoxime chloride will be place in an aluminium pan and hermetically sealed with an aluminum cover. Another empty aluminum pan with cover will be use as a reference. Nitrogen will be use to purge the sample chamber and calibrate temperature by using indium. Thermogram will be record during heating at a scanning rate 10°C/min from 25°C to 300°C.

5. Stability of freeze dried pralidoxime chloride.

Freeze dried pralidoxime chloride will be observed for its stability when the vials of freeze dried pralidoxime chloride are store in two desiccators by comparing the two humidity conditions. One of desiccators used an appropriate saturated salt solution, Potassium Iodide solution, to obtain about 70% relative humidity at 40°C. The other was the reference by using silica gel. The desiccators were placed in the controlled temperature oven at 40°C. Sample was pick to take a photograph and run XRPD one vial at each day of experiment to observed the impact of the humidity and temperature to the freeze dried characteristics.