CHAPTER III

MATERIALS AND METHODS

Materials

Two materials of anhydrous beclomethasone dipropionate used in this study were the products of Sigma Aldrich (Standard powder, analytical grade) and V&S Chemi Group Co. Ltd. (USP grade). The materials are identical in terms of x-ray powder diffraction patterns and differential scanning calorimetry thermograms (as shown in Appendix A). Dehydrated absolute ethanol used for the preparation of hydrated beclomethasone dipropionate was supplied from Labsan Asia Co. Inc. Deionized water (Ultrapure water) was used throughout the study.

Equipments

1. Analytical balance (Model AX204, Mettler Toledo, Switzerland)
2. Paddle blade stirrer (Model RW10R, Janke & Kunkel, IKA Labortechnik)
3. Convection oven (Model U30, Memmert, Germany)
4. Electronic thermo-hygrometer (Model JB 91B, DIGICON)
5. Infusion pump (Model STC-503, Terufusion, TERUMO)
6. Analytical screen sieving system (Model Asahi Sonic Sifer A1) with transformer (Model KD-100 Toyozami Dengenkiki Co Ltd.)
7. Differential scanning calorimeter (Model DSC 200C, NETZSCH, Germany)
8. Fourier transform infrared spectrophotometer (Model spectrum 2000, Perkin Elmer, Germany)

9. Particle size analyser (Mastersizer S long bed ver. 2.11, Malvern Instrument Ltd., Malvern, UK)
10. Scanning electron microscope (Model ISM-5410LV, Joel, Japan)
11. Ultrapure water equipped with filter system (Balston®, Balston Inc., USA) boost pump, option 3 water purifier, maximum ultrapure water and reservoir (Elga, USA)
12. X-ray powder diffraction spectrophotometer (Model PW3710 Diffractometer, Philips)
13. Thermogravimetry analyser (Model TGA7, Perkin Elmer,Germany)
14. Thermogravimetry analyser (Model STA 409C, NETZSCH, Germany)
15. UV-Visible spectrophotometer (Model UV-1601, Shimadzu®, Japan)
16. Water bath with shaker (Model 28L/B/SH/C, Poly Science®, USA)
17. Specific surface area analyzer (Model FlowSorb II 2300, Micromeritics®)
18. Optical microscopy (Model BH-2, Olympus®, Japan)

Methods

1. Preparation of hydrated becolmethasone dipropionate

To obtain the ration of water to absolute ethanol in crystallization of hydrated beclomethasone dipropionate, the solubility of beclomethasone dipropionate was determined in the solution profile of the ration of water to absolute ethanol at 8:2, 5:5 and 2:8 as indicated by a plateau of solubility curve at 25 °C.

Hydrate beclomethasone dipropionate was prepared by crystallization from binary mixture of solvent. Approximately 0.75g of anhydrous beclomethasone dipropionate was dissolved in 35 ml dehydrated absolute ethanol. Fifty milliliters of

deionized water was slowly added to the solution controlled by an infusion pump. The recrystallization was performed at 25 ± 1 °C. The crystals were collected on No. 1 Whatman® qualitative filter paper immediately after the addition of deionized water was done with the aid of Buchner funnel and were allowed to dry at ambient condition overnight.

Since rate of adding deionized water to the solution of beclomethasone dipropionate affects the size of crystals produced, various rates were applied in this study as 7, 23, 60, 120 and 300 ml/hr at a constant stirring rate of the paddle at 600 rpm.

Hydrate beclomethasone dipropionate crystals produced were then analyzed for its particle size employing laser particle size analyzer and scanning electron microscopy.

Three different sizes of hydrate beclomethasone dipropionate were desired for further study in observing their desolvation behavior. Crystals with different sizes were separated by passing through a series of small analytical sieves with apertures of 38, 75 and 150 μm, respectively. Sieving procedure started with 1 g of powder and sieved for 10 min. Particles/crystals with uniform size distributions were therefore collected, grouped and employed as representative of each particle size range.

Should the size of crystals were not appropriate for the study, they were subjected to gentle grinding with glass mortar and pestle. Ground sample was resuspended in deionized water for 72 hrs, collected on No.1 Whatman® qualitative filter paper and allowed to dry in ambient condition overnight.

As a result of this preparation, three groups of hydrated beclomethasone dipropionate crystals were obtained, i.e. intact crystals with approximately median size

of 430 μm, those with sizes between 38-75 μm (small) and those with sizes between 75-150 μm (medium). Sizes of each range of crystals were confirmed by laser particle size analyzer and SEM before being utilized in the future studies.

All samples were stored at room temperature in a humidity chamber partially filled with deionized water to maintain an approximate relative humidity of 95% and kept until further used.

2. Solid state characterization of hydrated beclomethasone dipropionate

2.1 Particle size analysis and morphology

Particle size analyses were performed twice, before desolvation process and once again after desolvation process. Particles were measured for their sizes by laser particle size analyzer and confirmed by scanning electron microscopy and light microscopy, which also revealed morphology of the particles. BET was employed to determine the specific surface areas of the particles.

2.1.1 Particle size analysis

The samples obtained were determined for their size and size distribution using laser particle size analyzer (Mastersizer®, Malvern Instrument Ltd.). The sample was dispersed in deionized water with sodium lauryl sulfate as a wetting agent. Measurement was made immediately after being stirred to avoid agglomeration of the particles and duration for each measurement was one minute. Each sample was determined for its size three times and the average of the three was recorded.

2.1.2 Scanning electron microscopy

Shape and size of hydrated beclomethasone dipropionate was studied by a scanning electron microscope (ISM-5410LV, JOEL®). The dry powder

was mounted onto metal stubs using a piece of double-slide conductive adhesive tape and vacuum-coated with gold. Electronphotomicrographs were taken. Shape and size of particles were revealed.

2.1.3 Specific surface area

Specific surface area of powder was determined by BET method, using a specific surface area analyzer (FlowSorb II 2300, Micromeritics®). Nitrogen gas was allowed to adsorb onto the surface of approximately 0.2 g of powder under normal atmospheric pressure. The specific surface area was calculated automatically by the instrument.

2.1.4 Light microscopy

The morphology of hydrated beclomethasone dipropionate was studied by light microscope (Model BH-2, Olympus®, Japan). The dry powder was mounted onto glass slide and photomicrographs were taken. Shapes of particles were revealed.

2.2 Thermal and spectroscopic characterization of hydrated beclomethasone dipropionate

Solid state characteristics of the hydrated crystals obtained were determined by the following methods.

2.2.1 Differential scanning calorimetry

Both anhydrous and hydrated forms of beclomethasone dipropionate were subjected to investigation for solid phase transformation by differential scanning calorimetry (DSC 200C, NETZCH). Approximately 2 mg of sample was weighed into an aluminum pan covered with a pierced lid. The container was placed on DSC holder and heated from 20 °C to 300 °C with an increment of 10 °C/min under nitrogen purge

at 10 ml/min. DSC thermograms showing phase transition and melting temperature were recorded.

2.2.2 Thermogravimetric analysis

The intact and ground hydrated beclomethasone dipropionate were determined for their water content using a NETZSCH STA 409 C thermogravimetric analyzer. Approximately 20 mg of the samples was accurately weighed into a aluminum oxide crucible. A heating rate of 10 °C/min was employed as the temperature ranged from 20-300 °C under nitrogen purge at the rate of 50 ml/min.

The TGA curve was often displayed as the amount of weight lost (percent of the initial mass). To determine the ratio of water to drug, the mass of each component must be calculated and the number of moles of water to drug was determined by the following equation (Morris and Rodriguez-Hornedo, 1993).

$$\frac{\text{mole water}}{\text{mole drug}} = \frac{\text{(grams water lost)/(18 g/mole)}}{\text{(grams sample-grams water lost)/(molecular weight of drug)}}$$

2.2.3 Qualitative x-ray powder diffraction analysis

X-ray powder spectra of anhydrous and hydrated forms were employed as the indicators of molecular arrangement of the samples. The x-ray powder diffraction patterns of anhydrous and hydrated forms obtained from this study were compared to those of standard powders established by the International Center for Diffraction Data (Nacheingtung, 1997).

The samples were packed onto a holder made of quartz plates containing rectangular window. The face of the holder was covered with a glass slide bonded with tape. Amount of crystals was loaded from the back, smoothed out and gently pressed using a microscope slide until it was at the same level as the frame of the

holder. The back of the holder was then covered with aluminum plate. Finally, the glass slide was removed to yield smooth surface.

The measurements were continuously scanned from 5° to 40° 2θ with a scanning rate of 0.04° 2θ per second at ambient temperature and atmosphere using 30 mA and 40 kV with Cuk_{α} radiation. Hence, diffraction patterns were obtained.

Since the particle size was one of the variables to be studied, all samples in this study were not ground before packing. Preferred orientation effect of the crystals was allowed to take place.

3. Dehydration process for particle size reduction of hydrated beclomethasone dipropionate

3.1 Solvent stability within the lattice of hydrated beclomethasone dipropionate

Isothermal dehydration was performed using a Perkin Elmer TGA 7 thermogravimetric analyzer. Approximately 2.0 mg of the samples was accurately weighed onto platinum pan under nitrogen purge at constant rate of 20 ml/min. The series of temperatures for isothermal scans of the three-size range were varied so that complete dehydration of each size could be established at a reasonable length of time. Isothermal gravimetric measurements of intact hydrated form were obtained at 85.5, 74.5, 65.0 and 56.0 °C. The ground samples of two sizes, 75-150 and 38-75 microns were subjected to series of isothermal gravimetric measurements at 85.5, 80.0, 74.5, 70.0 °C and 101.5, 90.5, 85.5, 80.0, 74.5 °C, respectively. At each temperature, the samples were measured in triplicate. For each experiment, the TGA furnace was rapidly heated to the required temperature at heating rate of 100 °C/min and the sample was maintained at that required temperature until dehydration was completed as indicated by constant net weight of the sample.

Analysis of dehydration data

Result of dehydration was analyzed based on the method of Taylor and York (1998). The fractional dehydration in the range 20-80% was calculated from the changes in weight at known time for the different experimental temperatures. These data were plotted according to the kinetic equations shown in Table 2 and the conformity of the plots was assessed by least squares correlation coefficients. The equations giving the best fits were then used to obtain kinetic parameters. The slopes of the linear plots at various isothermal temperatures correspond to the dehydration rate constants. The activation energy of the dehydration was estimated from the slope of an Arrhenius plot of the rate constants at various isothermal dehydration temperatures.

3.2 Structural stability (solid state stability) of dehydrated beclomethasone dipropionate

The interconversion between hydrated and anhydrous beclomethasone dipropionate was studied by placing known weight (approximately 0.2 g) of samples of different size range into 5 ml open glass vials. The vials were placed in a Memmert® oven of which temperature corresponded to the sample dehydration temperatures used in the previous experiments. The samples were periodically weighed and removed from the oven every 15 - 30 min. When obtaining the constant weight, the sample was collected to determine solid state transformation by x-ray powder diffractometer, particle size analyzer, scanning electron microscopy, specific surface area and light microscopy as described in previous Section 2.

From x-ray powder diffraction data, identification of interconversion phase was reported as 2θ where peaks appeared at 8.380° for the hydrated form and at 18.485° for the anhydrous form.