CHAPTER II

LITERATURE REVIEW

Beclomethasone dipropionate

Beclomethasone dipropionate, chemically known as 9α-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropionate ($C_{28}H_{37}ClO_7$), is a 21-carbon steriod. Its structure, as shown in Figure 1, resembles hydrocortisone.

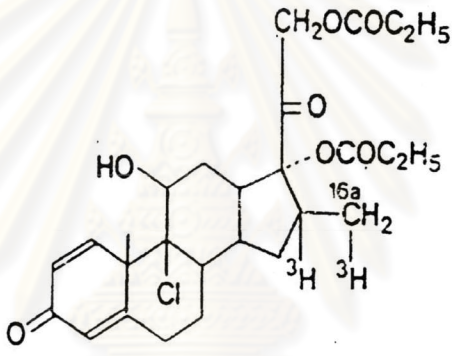

Figure 1 Chemical structure of beclomethasone dipropionate

Beclomethasone dipropionate melts at about 210 °C. It is practically insoluble in water, freely soluble in 96% ethanol and freely soluble in acetone and chloroform (British Pharmacopoeia Commission, 1993; Reynolds, 1994). Beclomethasone dipropionate appears in two forms, anhydrous and monohydrate. These two forms have the theoretical molecular weights of 521.05 and 539.07, respectively (United States Pharmacopoeia XXII, 1990). Anhydrous form is white to creamy white powder and has solubility of less than 5μg/ml in water and 22 mg/ml in alcohol at 25 °C. The

monohydrate is very slightly soluble in water and freely soluble in alcohol (McEvoy, 1999). The weight loss after drying of monohydrate can vary from 2.8% to 3.8% while the anhydrous form loses less than 0.5% of its weight (United States Pharmacopoeia XXII, 1990).

The monohydrate may be conventionally prepared by slowly adding water to a solution of beclomethasone dipropionate in a water miscible organic solvent such as methanol, ethanol, acetone and dioxan (Hunt and Padfield, 1989). After crystallization, the monohydrate may be isolated by filtration. It is then washed and dried in conventional manner such as air drying, drying under reduced pressure or in the presence of a sterile inert gas. Nevertheless, the monohydrate can be produced by comminuting beclomethasone dipropionate in water for 36 hours within a ball mill (Hunt and Padfield, 1989).

Other solvates of beclomethasone dipropionate have been reported with a variety of solvents. When the steroid is in contact with an alcohol containing 1 to 2 carbon atoms, such as isopropyl alcohol, solvate is formed (Jinks, 1989). Beclomethasone dipropionate can also form solvates with ethyl acetate, alkane having from 5 to 8 carbon atoms, di-isopropyl ether and chlorofluorocarbon (Finckenor, 1981; Neale and Taylor, 1997).

Beclomethasone dipropionate has been used to relieve the symptoms of bronchial asthma. It is an active component of Beclovent®, Becotide® and Vanceril® oral inhalation, and Vancenase® and Beconase® nasal inhalation (McEvoy, 1999). These drugs contain a microcrystalline suspension of the anhydrous form in their formulation and are commercially available.

Generally, beclomethasone dipropionate is micronized by conventional techniques, either a ball mill or fluid energy mill or ultrasonic means, into particles of

an appropriate size for endopulmonary or nasal inhalation, i.e., sizes ranging from 2-5 microns. The desired fraction can be separated out by air classification or sieving (Finckenor, 1980). However, when unsolvated drug is incorporated into the aerosol formulation containing halogenated hydrocarbons propellants such as trichloromonofluoromethane (propellant 11), dichlorotetrafluoroethane (propellant 114) and dichlorodifluoromethane (propellant 12), it is prone to the phenomenon of crystal growth and/or crystal agglomeration, where crystals of particle size larger than 20 microns are formed. Investigations have revealed that the large crystals are solvated with one of the propellants. Such crystals are unsuitable for inhalation since they can cause clogging of the metering valve in the aerosol, and are too large to penetrate far enough into the bronchial system (Page and Heggie, 1990; Finckenor, 1981).

To overcome the problem of crystal growth, micronizing a solvate and mixing it with the remaining aerosol propellants has been found useful and is claimed to inhibit crystal growth. Examples of this are di-isopropyl ether solvate and ethyl acetate solvate (Neale and Taylor, 1997). The hydrated becolmethasone dipropionate can be used intranasally as pump sprays containing the monohydrate in an aqueous vehicle. Effectiveness of beclomethasone dipropionate monohydrate is as equivalent as anhydrous formulation (McEvoy, 1999). Dry powder inhalation is another preparation using micronized beclomethasone dipropionate monohydrate in formulation as an active ingredient. It was found that the particle size of beclomethasone dipropionate monohydrate (1-10 microns) in such powder compositions remains substantially constant even after storage for extended periods (Hunt and Padfield, 1989). Although beclomethasone dipropionate is generally inhaled in aerosol form, inhalation capsule is another preparation that is available for patients who experience difficulty in using the

aerosol. Besides, beclomethasone dipropionate can be prepared as a cream or ointment containing 0.025% w/w used topically in the treatment of various skin disorders (Reynolds, 1994).

Mechanism of action and pharmacology of beclomethasone dipropionate

Beclomethasone dipropionate is an anti-inflammatory adrenal corticosteroid used for the treatment of seasonal or perennial rhinitis when conventional therapy with antihistamines and decongestants is ineffective. The exact mechanisms of actions of corticosteroids remains unknown but may involve reductions in the number of mediator cells and secretory response to cholinergic receptor stimulation (McEvoy, 1999) as well as suppression of inflammatory reactions in the bronchial walls. Even though the drug decreases bronchial hyperreactivity and has a direct bronchospasmolytic reaction, such effect occurs only when very high doses are administered (Mutschler and Derendorf, 1995).

Adverse effects of beclomethasone dipropionate are similar to those of corticosteroids in general. For examples, adrenal suppression may occur in some patients treated with long-term high dose (1,500-2,000 μg daily) inhalation therapy for asthma. It has been demonstrated that no significant suppression is likely to occur in the majority of patients when total daily dose is less than 1.5 mg (Reynolds, 1994; Law et al., 1986).

The incidence of *Candida* colonization in the oropharynx of *Candida albicans*-infected children taking inhaled beclomethasone dipropionate is reported (Shaw and Edmunds, 1986). Other adverse effects, due to acute respiratory infection, such as hoarseness, cough, dry mouth and sore throat are seen during the treatment periods (Silvasti et al, 1992).

Hydration and dehydration of solids

Solid chemical compounds can be divided according to their habit and crystal chemistry as shown in Figure 2. Habit refers to the description of the outer appearance of crystal, whereas the internal structure is the molecular arrangement within the solid.

Internal structure of a crystalline compound may contain a stoichiometric adduct, commonly referred to as 'solvate'. Solvate is a molecular complex that incorporates the crystallizing solvent molecules into specific sites within the crystal lattice. When the incorporated solvent is water, such complex is called a hydrate form (Fiese and Hagen, 1986).

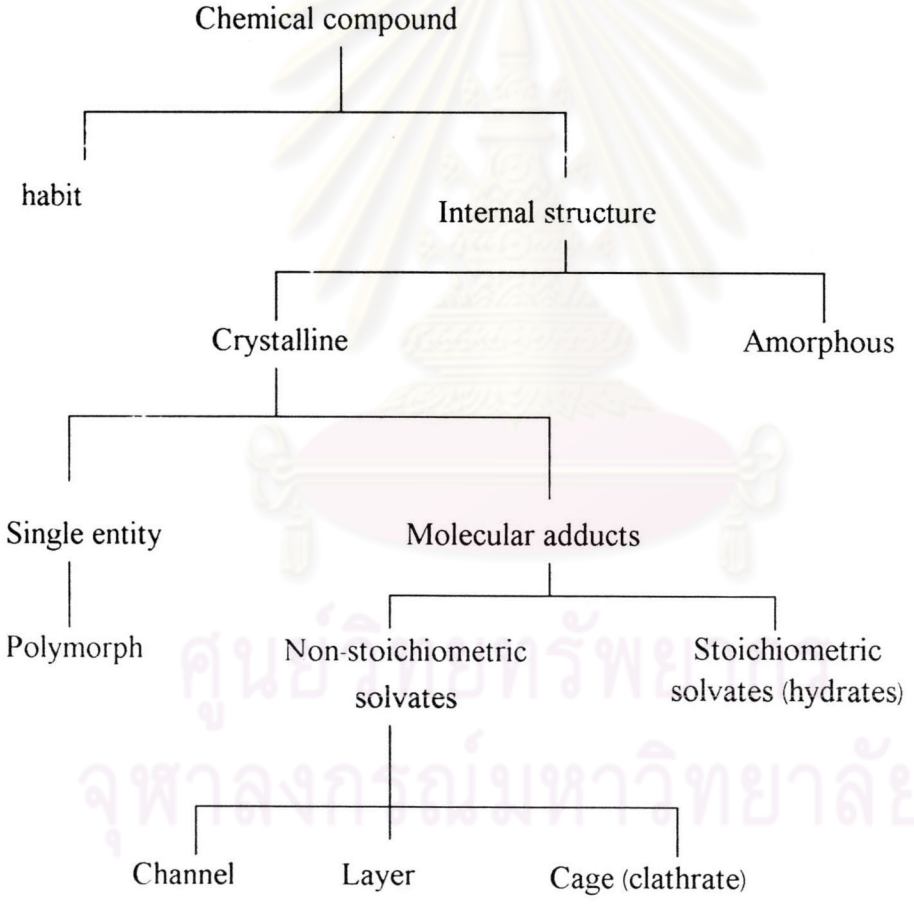

Figure 2 Solid state classification of chemical compounds

Because water molecule is small, it is particularly suited to fill structure voids. The multidirectional hydrogen capability of water is also ideal for linking a majority of drug molecules into stable crystal structure (Byrn et al., 1999).

Crystal habit

The crystal habit of pharmaceutical compounds has been used for purposes of evaluation of the morphology of pharmaceutical solid. If the environment of a growing crystal affects its external shape without changing its internal structure, a different habit results (Haleblian, 1975). A classification of observed crystal habits is proposed in Table1 and their habits are illustrated in Figure 3.

Table 1 Empirical classification of observed crystal habits (Adopted from Newman and Brittain, 1995)

Descriptor	Description
Acicular	Needlelike particle having a similar width and thickness. If the crystals are very thin, the term fibrous is used.
Columnar	Rod-like particle, having a width and thickness exceeding that of a needle-type particle. The term prismatic may also be used.
Blade	Long, thin, and flat particle, which can also be referred to as being lath-shaped.
Plate	Flat particles of similar length and width. These may also be denoted as being lamella or micaceous.
Tabular	Also flat particles of similar length and width, but possessing greater thickness than flakes.
Equant	Particles of similar length, width, and thickness.

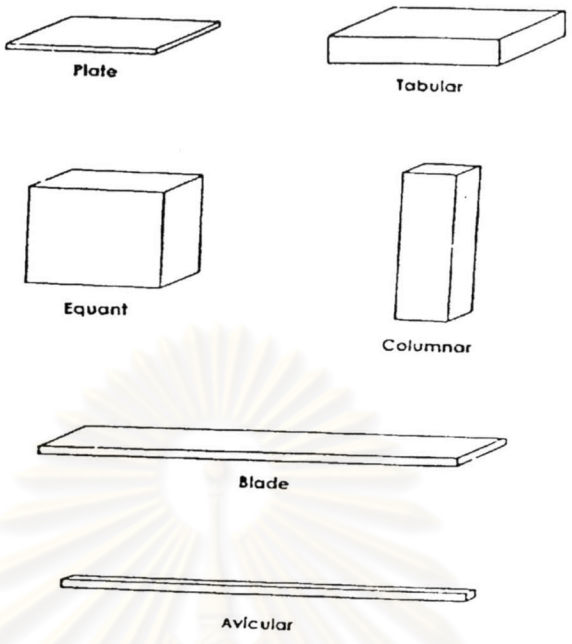

Figure 3 Classification of particulates according to their observed habits (Adopted from Newman and Brittain, 1995)

Deviation of crystal habits from the ideal may be observed, and such effects are normally due to the alteration in the crystallization condition. Factors affecting the crystal habits are, for examples, rate of deposition during growth, rate of deposition during growth, and composition of the crystallization process (Newman and Brittain, 1995).

As an instance, changing the crystallization solvent can lead to the changes of nitrofurantoin habits. The crystallization from formic acid resulted in tabular crystal habit whilst that from formic acid/water was needle-like. This habit modification was thought to be associated with preferential adsorption of the water molecules by polar crystal face, thus their growth was inhibited (Marshall and York, 1989). Moreover, increasing the phenytoin concentration during crystallization can lead to a

morphological change of the crystal from needles to elongated plates (Gordon and Chow, 1992).

Methods employed to obtain hydrate forms

The occurrence of hydrate crystals, onto which water may be adsorbed and/or absorbed in the bulk solid structure, is widespread but by no means universal among drug substances. Some classes of drug such as steroids, antibiotics and sulfonamides are particularly prone to form hydrate (Byrn et al., 1999).

Hydrate formation may occur during processes such as crystallization, lyophilization, wet granulation, aqueous film coating or spray drying. Moreover, hydrates can be produced by exposure to equilibrated moisture content for only a few days. Sometimes, hydrate can be accomplished by simply suspending anhydrous materials in distilled water or aqueous solution. For example, when dispersing anhydrous carbamazepine in distilled water at room temperature for 24-30 hours, hydrate form is produced. The slurry was filtered and stored under 55-60% relative humidity. These conditions assured that hydrate integrity was maintained (McMahon et al, 1996; Li et al., 2000).

Sheng and coworker found that eposartan mesylate dihydrate was accomplished in three ways. When suspending anhydrous drug in aqueous methane sulfonic acid for 2 hours or exposing to 98% RH for 15 days, the hydrate form can be produced. Another way is granulating anhydrous drug with binder solution as concentrated cornstarch paste using water. The experimental techniques for solid state characterization are used and confirmed that indeed it was the same phase of eposartan mesylate as dihydrate (Sheng et al., 1999).

Recrystallization in distilled water is another approach to produce hydrate form. When solution of a substance is evaporated, cooled or otherwise altered to reduce its solubility, supersaturation followed by nucleation will result in formation of hydrate crystals providing the form exists. Griesser and Burger (1995) found that caffeine 4/5 hydrate could be prepared by dissolving caffeine anhydrous in water at 50 °C and allowing it to slowly cool to room temperature. The crystals are then harvested. This method can also be used to produce particles of smaller sizes by simply stirring the solution during the crystallization process. In other instances, hydrates can be obtained from mixed aqueous solvent systems.

Often crystallizing a drug involves the use of good solvent to obtain a fairly concentrated solution. A miscible antisolvent chosen for its low solubility for the given drug is added to the solution to induce crystallization by forming a supersaturated mixture. In the most case, the solubility of the drug decreases smoothly during this process and a solvated crystal form is obtained (Byrn et al., 1999).

Urapidil does not form a solvate when recrystallized with 100% ethanol, 95% ethanol or denatured alcohol. On the other hand, when recrystalling it with water and ethanol in the ratio of 1:1 w/w, pentahydrates are formed. Furthermore, compounds of a given hydration state may crystallize in more than one form and lead to alterations of the crystals produced (Botha et al., 1988). One factor that many researchers consider is the activity of water (a_w) which can vary from zero to one with only slight change of water concentration in mixed solvent (Byrn et al., 1999). Nedocromil sodium trihydrate is crystallized by cooling a concentrated solution of its powder in a mixture of methanol and water at the ratio of 7:3 w/w (activity of water is 0.47). However, when crystallization is processed in the mixture of methanol and water of more than 0.9 water activity, heptahemihydrate is obtained (Khankari et al., 1998). It is the

activity of water in the medium that determines whether a given hydrate structure will form. Therefore, when substances that are capable of forming multiple hydrates are dissolved in water-immiscible solvents, different hydrates can be encountered if the water content of the system is not rigorously controlled.

Classification of hydrates

The crystalline hydrates produced may be classified into three classes according to their structures, which are discernable by the common analytical techniques available, single crystal x-ray diffractometry. A good classification system should direct the preformulation/formulation scientist to the characteristics of particular class that will help in identifying a new sample.

Class I Isolated lattice site: represents the structure where water molecules are isolated from direct contact with other water molecules by intervening drug molecules. An example is cephradine dihydrate of which pairs of water molecules arrange at interval in the lattice with carbonyl, carbonyl and amide groups on the two cephradine molecules (Morris and Rodrigues-Hornedo, 1993).

Class II Water forming lattice channels: the water molecules included in the lattice lie next to other water molecules of adjoining unit cells along an axis of the lattice, forming "channels" through the crystal. For instance, ampicillin trihydrate consists of eight unit cells and the water molecules line up along the *c* screw axis (Vippagunta et al., 2001).

Class III Ion–associated hydrate: in which the metal ions are coordinated with water, addresses the effect of the metal-water interaction on the structure of crystalline hydrate. This interaction can be quite strong. Forbes et al. (1992) found that it is more difficult to remove water from the magnesium and calcium salts of hydrated

p-aminosalicylic acid due to a stronger ion-dipole interaction for the divalent salts. Besides, crystallographic determination shows a greater bond length between metal ion and water oxygen of sodium salt as compared to those of magnesium and calcium salts. This study demonstrated the influence of crystal structure that may produce unusual physiochemical properties and behavior of crystalline hydrates.

Methods for the characterization of hydrates

Methods suitable for the characterization of hydrates may be classified as energetic (thermal and spectroscopic) or structural. Each method must yield information on the structure, composition or energy of association in the hydrate (Morris and Rodriguez-Hornedo, 1993).

1. Thermal methods of analysis

Measurements of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample. This requires that the operator interpret the observed events in a thermogram in term of plausible reaction process. The reaction normally monitored can be endothermic (melting, boiling, sublimation, vaporization, desolvation, solid-solid phase transitions, chemical degradation, etc.) or exothermic (crystallization, oxidative decomposition, etc.) in nature (McCauley and Brittain, 1995).

1.1 Differential scanning calorimetry (DSC)

The DSC experiment entails heating a reference pan and a pan containing the sample at identical rates of temperature change. The signal measured the difference in the amount of energy it takes to maintain the equal rate. If the sample absorbs heat during a phase transition (melting, vaporization), it takes up more energy to maintain the equality. Dehydration process takes energy to disrupt the association between

water molecules and drug molecules shown as an endothermic event. Among the most useful quantitative information available from DSC, heats of fusion for the drug or vaporization of water are calculated from the area of a DSC endotherm generated on a properly calibrated instrument. This is a direct measure of the reaction heat of the sample and the result is expressed in cal/s.g or J/s.g) (Morris and Rodriguez-Hornedo, 1993).

1.2 Thermogravimetric analysis (TGA)

Thermogravimetry is simply the measurement of the sample's weight change as a function of temperature or time (isothermal dehydration). The instrument is an extremely sensitive variable-temperature balance. Thermogravimetric analysis is restricted to studies involving either a mass gain or loss and it is most commonly used to study desolvation processes and compound decomposition. TG analysis is a very useful method for the quantitative determination of the total volatile content of a solid, and it can be used as an adjunct to Karl Fisher titration for the determination of moisture (McCauley and Brittain, 1995).

The isothermal rate of dehydration of drug crystals may also be measured by TGA. There are both energetic and geometric components of dehydration kinetics. In practice, the goal is to determine the "window" of time at given temperature that a hydrated drug is physically stable (Morris and Rodriguez-Hornedo, 1993).

The combination of TGA studies with DSC work can lead to unambiguous assignment of the observed thermal event. Examples are DSC and TGA profiles of unfractionated trehalose dihydrate at 10° C/min. The total weight loss is close to 9.5% w/w corresponding to the loss of two moles of water. It is evident that the rate of weight loss due to dehydration is most rapid over the temperature region

corresponding to the endotherms between 90 and 125 °C, linking them to the dehydration of the dihydrate crystal. The endotherm at 212 °C corresponds to the fusion of crystalline anhydrous trehalose as shown in Figure 4 (Taylor and York, 1997).

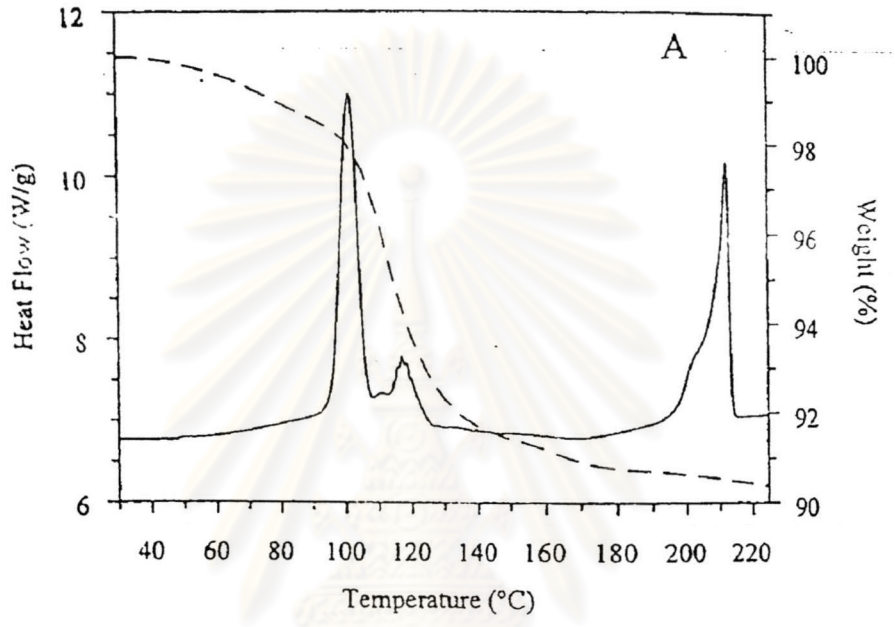

Figure 4 DSC and TGA (dashed line) profiles of unfractionated trehalose dihydrate at 10 °C min^{-1}

2. Structure determination

2.1 X-ray powder diffraction (XRPD)

X-ray powder diffractometry is widely used for the identification of solid phases. The technique can be used to identify the solvated and unsolvated (anhydrous) forms of a compound provided that the crystal lattices of the two are different (Suryanarayanan, 1995).

The x-rays are diffracted by the electrons around the atoms. The higher the electron density, the more intense the scattering. The relationship between the wavelength (λ), diffraction angle (θ), and the distance between the periodic planes of diffracting atom (d) is given by Bragg's law (Morris and Rodriguez-Hornedo, 1993).

$$n\lambda = 2d \sin\theta \quad (n = 1,2,3,....)$$

The powder x–ray diffraction pattern is a plot of peak intensities versus the diffraction angle (2θ). The patterns reflect the structure of the crystal but do not explicitly show the position of water, or any other molecules (Morris and Rodriguez-Hornedo, 1993).

The x-ray powder pattern of any crystalline form of compound is unique, making this technique particularly suited for the identification of different polymorphic forms of a compound. It is therefore used for the identification of hydrate form. For example, depending on the water vapor pressure, nedocromil sodium can exist as an anhydrous, a monohydrate or trihydrate. The x-ray patterns of these crystalline forms are different, thus x-ray powder diffraction pattern is used to characterize hydrate states of a compound (Khankari et al., 1998). Furthermore, the x-ray diffraction

patterns are used to detect changes in the hydrate due to hydration, dehydration or polymorphic transitions. This is accomplished by "peak matching" which may be done manually or by specially designed software. If virtually all of the peaks in the reference hydrate correspond to the peaks of unknown, these two crystals share a common structure. The relative peak intensity in each sample may vary, but the positions should agree at the 0.01° level. The appearance of new peak or disappearance of original peak after sample treatment is reasonable to suspect the phase change between the two samples (Morris and Rodriguez-Hornedo, 1993).

2.2 Morphology and particle size determination

2.2.1 Scanning electron microscopy

Evaluation of the morphology of a pharmaceutical solid is of extreme importance, since this property exerts a significant influence over the bulk powder properties of the material. Both light and electron microscopes have widely been used for the characterization of solid particles. The photomicrographs from transmission light microscopy showed that the nedocromil sodium monohydrate obtained by dehydration of the trihydrate at an elevated temperature (150 °C) or under low relative humidity at room temperature had the same external shape as the original trihydrate crystal, except that it was an opaque crystalline (Khankari et al., 1998).

In general, optical microscopy is more limited in the range of magnification suitable for routine work. Electron microscopy work can be preformed at extraordinarily high magnification level and the image obtained can contain a considerable degree of three-dimensional information. For instance, the electronphotomicrograph of the surface of theophylline hydrate is smooth while the anhydrous form after dehydration process showed high degree of roughness. In spite

of retaining the original external shape, dehydrated theophylline consists of agglomerates of smaller particles as shown in Figure 5 (Suzuki et al., 1989).

Usage of scanning electron microscope is beneficial in many ways but somewhat limited in another aspect because the information obtained is visual and descriptive, but usually not quantitative. When used in conjunction with other techniques, however, it becomes a powerful characterization tool for pharmaceutical materials.

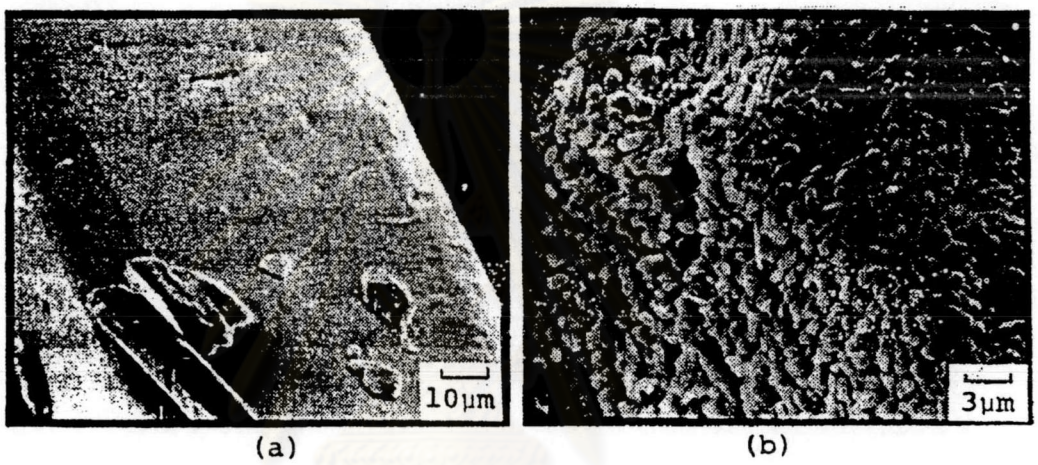

Figure 5 Scanning electronmicrographs of theophylline
(a) Theophylline monohydrate before dehydration
(b) Theophylline after dehydration

2.2.2 Light Scattering

The determination and control of particle size is often a necessity in pharmaceutical analysis. The size range and distribution of particles of a drug can influence its dehydration behavior of the hydrate form (Agbada and York, 1994).

Laser scattering particle size analyzer is equipment widely used to determine particle size distribution. Powder samples can be introduced into the laser in the dry form or, more commonly in suspension. It is important to choose a suspension medium to adequately disperse the sample, especially, the sample should not be soluble

in the intended medium used. Addition of surfactants may be necessary to facilitate dispersion and prevent flocculation, while a brief sonication is often required to reduce aggregation (Randall, 1995).

However, in all of the methods available for the physical characterization of solid materials, it is generally agreed that thermal analysis, crystallography microscopy and light scattering are the most useful tools for characterization of solvates. It is not overemphasized that the defining criteria for the compounds of pharmaceutical interest must be considered as sources of supporting and ancillary information. Each of these techniques alone, can not be taken as definitive proof for the existence of evidence.

Knowledge on hydration and dehydration of drug substance is essential in the development of stable formulation. When incorporating water into the crystalline lattice of anhydrate form, physicochemical and biological properties of hydrate are significantly different from those of the corresponding anhydrate. These properties include stability, solubility, dissolution rate, hygroscopicity, crystal habit (shape) (Agbada and York, 1994), crystal hardness, compactibility tablet behavior (Otsuka and Kaneniwa, 1988).

Shefter and Higuchi (1963) noted that the apparent dissolution rate and solubility of the anhydrous form of several drugs are greater than those of the hydrate form suggesting that the nucleation and formation of the more stable hydrate prevent realization of the true solubility of the metastable anhydrous form. Examples are those of glutethimide, theophylline, caffeine and fluorocortisone acetate. The higher thermodynamic activity of metastable anhydrous form is a major contributing factor causing the initially greater dissolution rate.

Occasionally, the binding capacity and flowability of α-D-glucose were increased after dehydration because of the differences in pore size distribution (Lerk et al, 1984). Moreover, many researchers are interested in the hydration and dehydration of disaccharides, e.g. sucrose (Saleki-Gerhardt and Zografi, 1994), trehalose (Taylor et al, 1998) and raffinose (Saleki-Gerhardt et al, 1995). These properties, hydration and dehydration, imply the potential of being a cryo-lyoprotectant during the process of freeze-drying.

Dehydration of a substance is also sensitive to environmental conditions and characteristics of the reactants. These include temperature (Fini et al., 1999), relative humidity and pressure (Griesser and Burger, 1995). In addition to the above factor, dehydration depends on the sample pre-history, sample weight and the geometry of the solid particle (Agbada and York, 1994).

In general, the process of dehydration can transform crystal hydrate into three types of crystallographic behavior. The solvate may exhibit the first type forming crystalline anhydrous. Examples of this type include theophylline monohydrate (Shefter et al., 1973) and mercaptopurine monohydrate (Niazi, 1977). The second type of behavior occurs when desolvation does not result in a change of crystal even though the solvent is lost. The result is a crystal with voids and cavities. The third type of behavior produces amorphous material or poor crystalline (Byrn et al., 1999).

Interestingly, subjecting raffinose pentahydrate samples to a vacuum oven of 30 °C for only 24 hours resulted in the removal of two out of five water molecules. X-ray powder diffraction measurements of the resulting samples showed the same integrated peak intensity as the original sample suggesting that the first two water molecules removed did not disrupt the crystal structure and most likely were the two loosely held waters. However, when increasing the temperature up to 60 °C, no change occurs in

the characteristic scattering angles of the pentahydrate form, but the integrated peak intensities at each angle are reduced with time until the sample appears completely as an amorphous form as shown in Figure 6. Because no new peak associated with any anhydrous crystal form appears, it would seem that the heat treatment of raffinose pentahydrate at this temperature and intermediate times can lead to various mixtures of amorphous and crystal pentahydrate. The implications of this observation would be important if the variable solid-state molecular disorder introduced could lead to enhanced chemical or physical instability (Saleki-Gerhardt et al., 1994).

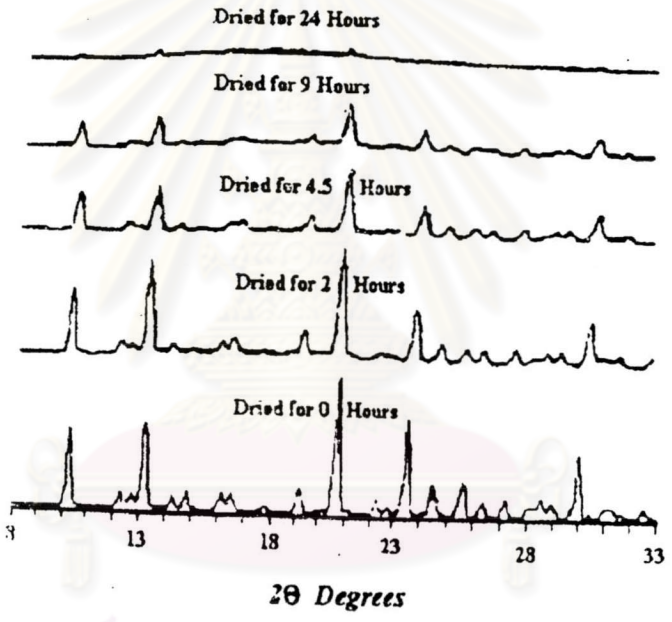

Figure 6 X-ray powder diffraction for raffinose pentahydrate crystals subjected to vacuum drying at 60°C for different time periods (Adopted from Saleki-Gerhardt et al., 1994)

A dihydrate of diclofenac N-(2-hydroxyethyl) pyrrolidine can transform to anhydrous form even at low temperature of 40 and 50 °C. Dehydration is completed in 72 and 10 hours, respectively. Moreover, anhydrous form can occur when keeping its hydrate form in a desiccator over silica gel at room temperature for one week. The thermal analysis, observed by differential scanning calorimetry, showed that the anhydrous form had a higher melting point as compared to the hydrate form. This indicated that the transformation of a less stable hydrate form to a more stable anhydrous form might occur when stored at low temperature and relatively low humidity condition (Fini et al., 1999).

Thermogravimetric curve at temperatures ranging from 60-80 °C showed a higher dehydration rate of particles of smaller size. An example is of theophylline monohydrate powder (<150 μm), prepared by gently grinding with pestle and mortar, as compared to those of larger particles (>500 μm). Observation was made that the activation energy decreases with a decrease in particle size. It was suggested that this could due to the enhanced dehydration from the greater surface to mass/volume ratio of smaller particles, which in turn promotes the removal of the water (Agbada and York, 1994).

Grinding generally affects the crystallinity of a compound. The interaction between water and drug molecules in crystals subjected to such forces has been extensively studied. Kitamura et al. (1989) found that crystalline of cefixime trihydrate was changed to a non-crystalline solid after 4 hours of grinding in a ball mill and dehydration temperature of ground cefixime trihydrate also lowered with increasing grinding time. Calculated activation energies for dehydration of intact and that of 4-hour ground samples were 72.4 and 67.5 kcal/mol, respectively. These clearly show

that the grinding process weakened the bonding force between water molecule and cefixime molecule. That is, the ground samples became less stable as the grinding effects. This phenomenon is considered to be due to a greater freedom of movement of water molecules, which are sufficiently free to participate in a solid-state hydrolytic reaction in the impaired lattice induced by grinding (Takahashi et al., 1984; Nakagawa et al., 1982).

Occasionally, the size reduction was produced by solvation and desolvation methods. Sekiguchi et al (1964) reported that the formation and decomposition of griseofulvin chloroformate play an important role in reducing the particle size of griseofulvin. When the drug was treated with chloroform, disintegration of the rigid crystals occurs in a little while as a result of conversion of crystal structure. However, after being treated furthermore by desolvation in a vacuum at high temperature of 150 °C, a rearrangement of crystal to ordinary compound occurred and obviously the particle size was reduced at this stage. Although the particle size reduction was accomplished, but also in the larger crystals, this process was hindered by the difficulty of migration of the solvent molecules from the crystal nuclei. In 1968, Sekiguchi and co-workers, using differential scanning calorimetry (DSC) and gas evolution analysis (GEA), found that the heat of solvation and activation energy of desolvation reaction of griseofulvin chloroformate were 6.4 ± 0.5 and 20 ± 1.0 kcal/mol, respectively. And in order to find the limit of size reduction by the methods of solvation and desolvation, the BET gas adsorption method was used to investigate on surface area the drug after repeated solvation and desolvation. It is noted that the size reduction was accomplished by one cycle of solvation and desolvation, therefore, further application is unnecessary. Moreover, the same mechanism of particle size reduction was applied to some other solvated compounds such as glucuronamide, potassium glucuronate,

sodium glucuronate isoniazone, citric acid, picric acid and sulfathiazole. Among the above desolvated compounds, surface area of solvated form changes from 0.2 m^2/g to 2.0-3.5 m^2/g after desolvation except in the case of citric acid where its surface area of the dehydrate compound is only 0.89 m^2/g (Sekiguchi et al., 1968).

Kinetics of desolvation

In general, the kinetics of solid-state decomposition reaction of pharmaceuticals has been studies to make shelf-life predictions and to generate preliminary expiration data. The aim of most of these studies was to obtain an equation that provides an adequate fit for plots of rate versus time and to elucidate the molecular details of the solid state reaction (Byrn et al., 1999). Occasionally, the work has been done to study the dehydration behavior and kinetics of solvate or hydrate form, which is the basis information required for predicting the stability and other physicochemical properties (Sheng et al., 1999). However, in the desolvation process, the physical transformation is of high concern. Theophylline monohydrate transforms directly to a crystalline anhydrous form with apparent zero order kinetics and the loss of water from ampicillin trihydrate results in amorphous state (Shefter et al., 1973).

Usually, analysis of data from a solid-state reaction kinetic study begins with plotting the fraction decomposition (α) versus time (t). To determine rate constants, k, kinetic equations are chosen to fit the initial data; the slope of the linear plots at the various isothermal dehydration temperatures correspond to the dehydration rate constants. The activation energy of the dehydration was obtained by estimation from an Arrhenius plot of the rate constants and temperatures (Taylor and York, 1998). The fact that more than one equation fits the data indicates that solid-state kinetics data cannot be used to identify the mechanism of a solid-state reaction. Nevertheless,

it may be important for determination of the activation energy to select the proper kinetic equation (Byrn et al., 1999).

Kinetics study on the isothermal thermogravimetric analysis showed that the dehydration mechanism and activation energy of thophylline monohydrate depended on both the particle size and sample weight. For samples of small weight, dehydration followed Avrami-Erofeev (n = ¼) equation regardless of the particle size. However, when sample weight was large, particles of small size followed two-dimensional phase boundary equation while unfractionated sample followed Avrami-Erofeev (n = ¼) mechanism. These analyses suggest that the mechanism of dehydration of hydrate could be significantly influenced by sample pre-history such as particle size, sample weight, crystal defects and surface characteristics. Therefore, meaningful comparisons of kinetics parameters should relate data acquired under similar experimental conditions (Agbada and York, 1994).

There are many kinetics equations derived from different reaction models and concepts. They are currently used in assessing kinetic parameter, especially to determine the activation energies of solid-state reactions.

Equations for kinetic analysis of solid-state reactions

1. Reactions involving nucleation

1.1 The Prout-Tompkins equation

The rate of reaction is presumably controlled by nuclei that grow linearly branch into chain and are terminated when the chains of the growing crystals come in contact with one another (Byrn et al., 1999).

$$ln(\alpha/1-\alpha) = kt$$

where α = fraction decomposed
k = rate constant
t = time

No solid has a smooth surface, there are always surface imperfections or they could be crystal defects. These sites are more energetic than the remaining sites. It is assumed that decomposition is more likely to occur at such "activated" site. Once a molecule decomposes at an activated site, it changes its geometry; hence, the neighboring molecules are more likely to decompose. There will then be a chain or plane of activated molecules forming as shown in Figure 7 (Carstensen, 2001).

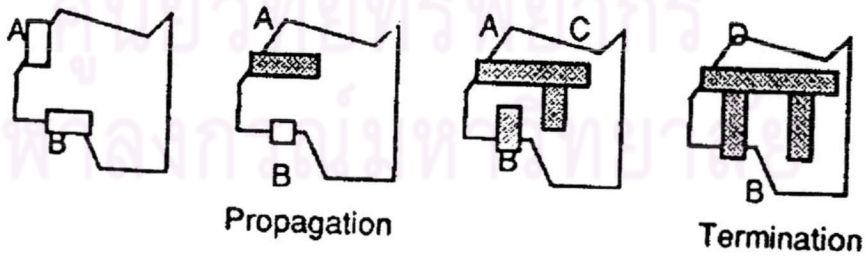

Figure 7 Propagation of active site chains. A propagates and becomes AC while B stares. After that, B branches with AC and terminates. (Adopted from Carstensen, 2001)

1.2 The Avrami-Erofeev equation

The rate of the reaction is presumably controlled by random nuclei that grow in three-dimensional directions and are able to ingest other nuclei (Byrn et al., 1999)

$$[-\ln(1-\alpha)]^n = kt$$

where n = 1/4, 1/3, 1/2, 2/3 and 1
(n being the proportion of the numbers of nuclei)
α = fraction decomposed
k = rate constant
t = time

The Avrami-Erofeev model assumes that volumes within the solid at a given time (t) are activated, and that decomposition may occur in these areas as shown in Figure 8 (Carstensen, 2001).

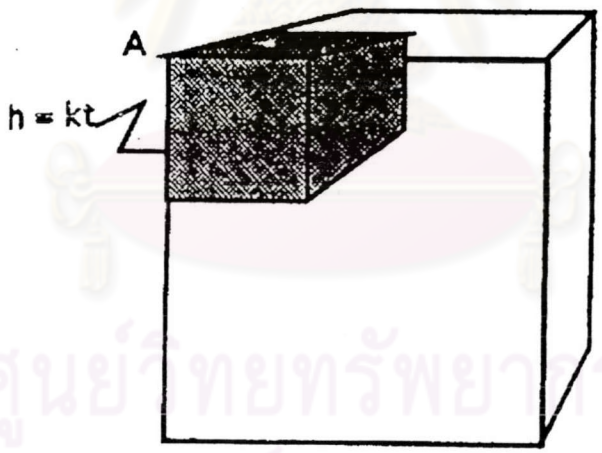

Figure 8 Schematic for approximate Avrami-Erofeev model
(Adopted from Carstensen, 2001)

2. Reactions controlled by phase boundaries

If the solid state reaction is assumed to be controlled, not by the formation of nuclei, but rather by the advancement of phase boundaries from the outside of a crystal inward, then a different series of equations can be derived (Byrn et al., 1999).

2.1 One-dimensional advancement of a phase boundary

The reaction is assumed to proceed along one direction. The rate is a function of time. Thus, a zero-order rate equation is applied.

$$1- \alpha = kt$$

where α = fraction decomposed
k = rate constant
t = time

2.2 Two-dimension advancement of a phase boundary

The reaction is assumed to grow from the surface of a circular disk or a cylinder. This equation is also known as the controlling area (disc, cylinder, or rectangle) equation.

$$1- (1- \alpha)^{1/2} = kt$$

where α = fraction decomposed
k = rate constant
t = time

2.3 Three-dimensional advancement of a phase boundary

The reaction is assumed to proceed from the surface of a sphere inward.

$$1- (1-\alpha)^{1/3} = kt$$

where α = fraction decomposed
k = rate constant
t = time

3. Reaction controlled by diffusion

Obviously, primarily reaction involving gaseous starting materials or products should be treated as diffusion – controlled reactions (Byrn et al., 1999).

3.1 One-dimensional diffusion

The rate of the reaction is controlled by a one-dimensional diffusion process.

$$\alpha^2 = kt$$

where α = fraction decomposed
k = rate constant
t = time

3.2 Two-dimensional diffusion

The rate of the reaction is controlled by two-dimensional diffusion from the surface of a circular disk or cylinder, then equation can be derived.

$$(1-\alpha)\ ln\ (1-\alpha)+\alpha = kt$$

where α = fraction decomposed
k = rate constant
t = time

3.3 Three-dimensional diffusion

If the reaction is assumed to be controlled by diffusion from the surface of a spherical particle, then equation can be derived (Ginstling-Brounshtein equation).

$$1- 2/3\alpha - (1-\alpha)^{2/3} = kt$$

where α = fraction decomposed
k = rate constant
t = time

A simplified version of Ginstling-Brounshtein equation is known as the Jander equation.

$$[1-(1-\alpha)^{1/3}]^2 = kt$$

where α = fraction decomposed
k = rate constant
t = time

Equations based on the concept of order of the reaction

Solution reactions are routinely analyzed in terms of equations based on the concept of order, and often the order of a reaction is used to sign insight into the molecularity of a reaction. Since the concept of molecularity of reaction is not as well defined for solid-state reaction, kinetic equations based on order are not as widely used. Nevertheless, data are sometimes analyzed in these terms (Byrn et al., 1999).

1. Zero–order equation

$$1 - \alpha = kt$$

where α = fraction decomposed
k = rate constant
t = time

2. First–order equation

$$\ln(\alpha) = kt$$

where α = fraction decomposed
k = rate constant
t = time

3. Second–order equation

$$1/(1-\alpha) = kt$$

where α = fraction decomposed
k = rate constant
t = time

Several such equations, some of them already alluded to, are listed in Table 2.

Table 2 Kinetic equations of most common mechanisms of solid state decompostions (compiled from Byrn, 1999)

Equation	Rate-controlling process
	Reactions involving nucleation
$\ln(\alpha/1-\alpha) = kt$	• Linearly growing nuclei *Prout-Tompkins*
$(-\ln(1-\alpha))^n = kt$	• Random nuclei *Avrami-Erofeev* n = 1/4, 1/3, 1/2, 2/3, 1
	Reaction controlled by phase boundaries
$1-\alpha = kt$	• One-dimensional phase boundary (zero-order mechanism)
$1-(1-\alpha)^{1/2} = kt$	• Two-dimensional phase boundary
$1-(1-\alpha)^{1/3} = kt$	• Three-dimensional phase boundary
	Diffusion controlled reaction
$\alpha^2 = kt$	• One-dimensional diffusion
$(1-\alpha)\ln(1-\alpha)+\alpha = kt$	• Two-dimensional diffusion
$1-2/3(\alpha)-(1-\alpha)^{2/3} = kt$	• Three-dimensional diffusion (Ginstling-Brounshtein equation)
$(1-(1-\alpha)^{1/3})^2 = kt$	• Three-dimensional diffusion (Jander equation)
	Other equations
$\ln(\alpha) = kt$	• First order
$1/(1-\alpha) = kt$	• Second order

After all the kinetic equations are fitted, only the one with the best correlation coefficient should be chosen for the determination of activation energy. The activation energy can be obtained by the Arrhenius equation.

$$k = Ae^{-Ea/RT}$$

where k is the rate constant, Ea is the activation energy, T is temperature in Kelvin, R is the rate constant and A is proportionality constant. By plotting the logarithm of the rate constants against $1/T$, the activation energy can be calculated from the slope of the plot. The activation energy can be used to calculate the rate constant at the labeled storage conditions.