

**SOLID PHASE TRANSFORMATION AND STABILITY OF
CARBAMAZEPINE-ADIPIC ACID CO-CRYSTAL**

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ABSTRACT

Co-crystal is a mixed crystal or crystal that contains two or more different molecules. In this study, the solid phase transformation of CBZ-AA co-crystal and solubility of carbamazepine (CBZ) and adipic acid (AA) is examined. In this dissertation, the solubility of CBZ and AA via HPLC and gravimetric methods are reported. On the other hand, the study of solid phase transformation is planned to proceed via slurry co-crystallization method. The result shows the solubility of CBZ and AA via gravimetric and HPLC method is increased as the temperature increased from 25 °C to 50 °C. TGA analysis shows the weight changed of CBZ is 96.21 % at decomposed temperature of 250 °C and 94.47 % for AA at 220 °C decomposed temperature. The wavenumbers corresponding to CBZ and AA is obtained from FTIR analysis. Due to time limitation, the phase transformation of CBZ-AA co-crystal is suggested to be continued by another student in the future.

ABSTRAK

Ko-kristal adalah kristal yang bercampur atau kristal yang mengandungi dua atau lebih molekul yang berbeza. Dalam kajian ini, transformasi fasa pepejal CBZ-AA bersama kristal dan keterlarutan carbamazepine (CBZ) dan asid adipic (AA) diperiksa. Dalam disertasi ini, keterlarutan CBZ dan AA melalui kaedah HPLC dan gravimetrik dilaporkan. Sebaliknya, kajian transformasi fasa pepejal merancang untuk meneruskan melalui kaedah buburan bersama-penghabluran. Hasilnya menunjukkan keterlarutan CBZ dan AA melalui gravimatrik dan kaedah HPLC meningkat apabila suhu meningkat daripada 25 ° C hingga 50 ° C. Analisis TGA menunjukkan berat yang berubah daripada CBZ adalah 96,21% pada suhu lebur sebanyak 250 ° C dan 94,47% untuk AA pada suhu 220 ° C. Wavenumbers sepadan CBZ dan AA diperolehi daripada analisis FTIR. Oleh kerana had masa, transformasi fasa CBZ-AA ko-kristal dicadangkan untuk diteruskan oleh pelajar lain pada masa akan datang.

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CHAPTER 1

INTRODUCTION

1.0 Research background

Co-crystals have recently gained attention as attractive alternate solid forms for drug development. A pharmaceutical co-crystal is a single crystalline homogenous phase consisting of a drug and excipient or another drug. (Trask et al., 2005; Etter et al., 1990; Pedireddi et al., 1996; Trask et al., 2004; Morissette et al., 2004; Nehm et al., 2005; Rodriguez et al., 2006; Crowley et al., 2002). The different components in the co-crystal are neutral in nature when compared to salts that have ionized components. (Etter et al., 1990; Morissette et al., 2004).

Co-crystals, multiple component crystals, often rely on hydrogen-bonded assemblies between neutral molecules of the active pharmaceutical ingredient (API) and other components with well-defined stoichiometries (Etter 1991; Caira 1992; Desiraju 1995; Nangia and Desiraju 1998; Byrn, Pfeiffer et al. 1999; Aakeroy, Beatty et al. 2002; Almarsson and Zaworotko 2004; Wenger and Bernstein 2008). Therefore, co-crystals increase the diversity of solid-state forms of an API even for non-ionizable APIs, and enhance pharmaceutical properties by modification of chemical stability, moisture

uptake, mechanical behavior, solubility, dissolution rate, and bioavailability (Remenar, Morissette et al. 2003; Rodríguez-Spong, Zocharski et al. 2003; Childs, Chyall et. al., 2004; Rodríguez-Spong, Price et. al. 2004; Zocharski, Nehm et. al. 2004; Rodríguez-Spong 2005; Trask, Motherwell et al. 2005; Nehm, Rodríguez-Spong et. al. 2006).

Families of API co-crystals are being designed and prepared by applying molecular recognition, thermodynamic, and kinetic principles to build hydrogen-bonded molecular assembly of multiple components. Co-crystals are gaining much interest because the resulting new crystal forms of APIs have different physical and chemical properties compared to the original API.

1.1 Co-crystal

In 1984, Kitaigorodski defined co-crystal as “a mixed crystal or crystal that contains two different molecules”. An alternative approach that arises conceptually from applying the concepts of supra-molecular chemistry and crystal engineering is that a co-crystal is the consequence of a molecular recognition event between molecular species. (Dunitz and Desiraju, 2003).

Co-crystal incorporates pharmaceutically acceptable guest molecules into a crystal lattice along with the API. Co-crystals are homogeneous solid phases containing two or more neutral molecular components in a crystal lattice with defined stoichiometry, which are solids at room temperature and are held together by weak interactions, mainly hydrogen bonding (Aakeroy and Salmon, 2005). Co-crystal has regained attention as attractive alternate solid forms for drug development. Physicochemical properties of pharmaceuticals can be improved by obtaining co-crystal

using co-crystallization (Shan and Zaworotko, 2008; Jones et al., 2006). In their pure states generally co-crystal are solids at room temperature and by convention, normally these also excludes salts. Co-crystal can have different properties, crystal structures, different intermolecular packing patterns, and often exhibit widely different physical properties than their pure components. (Miroshnyk et al., 2009; McMahon, 2006).

1.2 Objective

Based on research background mentioned above, the objectives of this study are:

- To measure the solubility of CBZ and AA.
- To study the phase transformation of CBZ-AA co-crystal formation.

1.3 Scope of research

The scopes of research are listed as below:

- Measure solubility of CBZ and AA via gravimetric and HPLC method.
- Manipulate the AA to CBZ ratio to measure co-crystal transformation based on solubility data obtained.

1.4 Significance of research

This study were carried out to measure the solubility of CBZ and AA, later to be used to manipulate the AA to CBZ ratio in order to study the co-crystal transformation region. The transformation study is important because we can determine the suitable concentration range of CBZ and AA to produce co-crystal.

1.5 Report layout

This report contains five main chapters to distribute the whole report accordingly. In the first chapter, explained the introduction which gave the briefing about the project. The second chapter contains literature review based on crystallization and co-crystal. The third chapter explained the methodologies of the experiment and fourth chapter contained results and discussions. Finally, fifth chapter contains with conclusions and recommendations.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

2.1.1 Crystallization

Crystallization is a process where solid particles are formed from homogeneous phase. Crystallization process can occur in the freezing of water to form ice, in the formation of snow particles from a vapor, in the formation of solid particles from a liquid melt, or in the formation of solid crystals from a liquid solution (Geankoplis, 2003). From the last process that mentioned above, crystallization from a solution is the most important one commercially. In crystallization the solution is concentrated and usually cooled until the solute concentration becomes greater than its solubility at that temperature. Then the solute comes out of the solution, forming crystals of approximately pure solute (Geankoplis, 2003).

There are seven classes of crystal that can be classified, depending upon the arrangement of the axes to which the angles are referred:

- 1) Cubic system. Three equal axes at right angles to each other.
- 2) Tetragonal system. Three axes at right angles to each other, one axis longer than the other two.
- 3) Orthorhombic system. Three axes at right angles to each other, all of different length.
- 4) Hexagonal system. Three equal axes in one plane at 60° to each other, and fourth axis at right angle to this plane and not necessarily at the same length.
- 5) Monoclinic system. Three unequal axes, two at right angles in plane and a third at some angle to this plane.
- 6) Triclinic system. Three unequal axes at unequal angles to each other and not 30° , 60° , or 90° .
- 7) Trigonal system. Three equal and equally inclined axes.

2.1.2 Co-crystallization

Co-crystallization is a result of competing molecular associations between similar molecules, or homomers, and different molecules or heteromers. Hydrogen bonds are the basis of molecular recognition phenomena in pharmaceutical systems and are responsible for the generation of families of molecular networks with the same molecular components (single component crystals and their polymorphs) or with different molecular components (multiple component crystals or co-crystals) in the crystalline state (Jayasankar et al., 2006)

2.1.3 Physical properties of co-crystals

As there is no new covalent bond formed in a co-crystal compared to its mother molecules, the chemical properties of the original compounds will be well preserved. However, the crystal structure of a co-crystal is totally different from the crystals of its mother compounds. Therefore a co-crystal has different physical properties from its mother crystals (as shown in Figure 2.1).

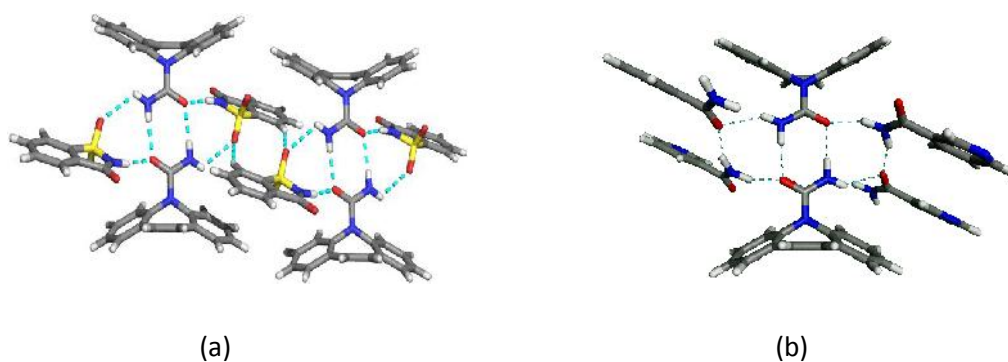


Figure 2.1: (a) CBZ-saccharin co-crystal, (b) CBZ-nicotinamide co-crystal. (Zaworotko, 2003 and Rodriguez-Hornedo et al., 2003).

2.2 Crystallization process

The crystallization process consists of two major events, nucleation and crystal growth. Nucleation is the step where the solute molecules dispersed in the solvent start to gather into clusters, on the nanometer scale (elevating solute concentration in a small region), that become stable under the current operating conditions. The crystal growth is the subsequent growth of the nuclei that succeed in achieving the critical cluster size. Nucleation and growth continue to occur simultaneously while the supersaturation

exists. Supersaturation is the driving force of the crystallization; hence the rate of nucleation and growth is driven by the existing supersaturation in the solution.

2.2.1 Solubility

Solubility studies were conducted by equilibrating excess solids in appropriate solvent mixtures for a minimum of 3d. A single HPLC method using YMC-ODS-AM column was developed and run on an Agilent 1100 system to quantify both CBZ and AA concentrations. Solubility data obtained from the HPLC method were compared and shown to be similar to the data obtained from the gravimetric analysis of the solubility samples.

2.2.2 Nucleation

Nucleation is a physical reaction which occurs when components in a solution start to precipitate out, forming nuclei which attract more precipitate. This physical reaction is the basis for a variety of manufacturing processes and interesting natural phenomena.

Nucleation can often be induced by agitation, mechanical shock, friction and extreme pressure within solutions and melts. (Young, 1911 and Berkeley, 1912). The erratic effects of external influences such as electric and magnetic field, spark discharges, ultra-violet light, X-ray, sonic and ultrasonic irradiation have also been studied over many years (Khamskii, 1969) but so far none of this methods has found any significant application in large-scale crystallization process.

There have two behavior term will be defined in nucleation that it primary and secondary. Primary term is for all cases of nucleation in systems that do not contain crystalline matter. In primary, the cases is divide into two part that called homogeneous (spontaneous) and heterogeneous (induced by foreign particle). On the other hand, nuclei are often induced by crystals present in a supersaturated system; this behavior is been called secondary.

2.2.3 Crystal growth

As soon as stable nuclei have been formed in a supersaturated or super cooled system, they begin to grow into crystals of visible size. There have been many proposed for mechanisms of crystal growth such as surface energy theories, diffusion theories, adsorption layer theories, and kinematic theories.

The surface energy theories are based on the postulation that the shape a growing crystal assumes is that which has a minimum surface energy. In 1878 Gibbs (1948) suggested that the growth of a crystal could be considered as a special case of this principle; the total free energy of a crystal in equilibrium with its surroundings at a constant temperature and pressure would be minimum for a given volume.

The diffusion theories presume that the matter is deposited continuously on a crystal face at a rate proportional to the difference in concentration between the point of deposition and the bulk of the solution. The origin of the diffusion theories dates back to the work of (Noyes and Whitney, 1897) who considered that the deposition of solid on the face of a growing crystal was essentially a diffusion process. They also assumed that crystallization was the reverse of dissolution and that the rates of both processes were

governed by the difference between concentration at the solid surface and in bulk of the solution.

The adsorption layer theories define that concept of a crystal growth mechanism base on the existence of an adsorbed layer of solute atoms or molecules on a crystal face (Volmer, 1939).

Kinematic theories is about two processes involved in the layer growth of crystals, the generation of steps at some source on the crystal face followed by the movement of layers across the face. Consideration of the movement of macro steps of unequal distance apart (BCF theory considers a regular distribution of monatomic steps) to develop a 'kinematic' theory of crystal growth (Frank, 1958).

2.3 Development of co-crystal

2.3.1 Co-crystal screening method

Co-crystal screening methodology has advanced from being empirically based to a more efficient and rational basis (Childs et al., 2008). The method can be broadly being categorized as solid-based and liquid based (Table 2.1). While solid-based methods often rely on the stoichiometric ratio of the reactants for co-crystal formation, the liquid-based methods can be either stoichiometric such as (slow evaporative crystallization, spray drying) or non-stoichiometric such as (slurry and reaction crystallization) (Alhalaweh and Velaga, 2010; Rodriguez-Hornedo et al., 2006; Zhang et al., 2007).

Table 2.1: Shows the liquid-based method and solid-based method

| Liquid-based methods | Solid-based methods |
|-----------------------------|----------------------|
| Slow evaporation | Melt crystallization |
| Slurry conversion | Solid-state grinding |
| Reaction co-crystallization | Melt extrusion |
| Cooling crystallization | |
| Liquid-assisted grinding | |
| Supercritical fluids | |
| Spray drying | |

2.3.2. Co-crystallization techniques

2.3.2.1 Slow evaporation.

This technique is the simplest way to grow crystals and works best for compounds which are not sensitive to ambient conditions in the laboratory. The solution of the compound is been prepared in a suitable solvent until the solution is saturated or nearly saturated. After that, the solution is transferred to a clean crystal growing dish and cover. The covering for the container should not be air tight. Aluminium foil with some holes poked in it works well, or a flat piece of glass with microscope slides used as a spacer also will do the trick. The container is placed in a quiet out of the way place and let it evaporate. This method works best where there is enough material to saturate at least a few milliliters of solvent (Jones, 1981).

2.3.2.2 Slow cooling.

A saturated solution of the compound is prepared where the solvent is heated to just its boiling point or a just below it. Transferred the solution to a clean large test tube and placed it into Dewar flask in which hot water (heated to a temperature of a couple of degrees below the solvent boiling point ($<100^{\circ}\text{C}$)). The water level should exceed the solvent level in the test tube. Stopper the Dewar flask with a cork stopper and let the vessel sit for a week. A more elaborate version of this involves a thermo stated oven rather than a Dewar flask (Jones, 1981).

2.3.2.3 Slurry.

API component or co-former solid it's been dissolved into ethanol solvent till equilibrium of those mixed is achieved. Then, solution is left for 3 days (72 hours) with stirring. After 3 days, the exceed solution is filtrate by using syringe, syringe filter and needle into volumetric flask. The filter solution is around 4 ml. 1ml from it is dilute to test its solubility in High Performance Liquid Chromatography (HPLC) and another 3ml from it using in gravimetric solubility test. (Takata et al., 2008)

2.3.2.4 Grinding.

When preparing co-crystals, the product obtained from grinding is generally consistent with that obtained from solution. This may indicate that hydrogen-bond connectivity patterns are not idiosyncratic or determined by non-specific and unmanageable solvent effects or crystallization conditions. Nevertheless there are exceptions. Whilst many co-crystal materials can be prepared from both solution growth and solid-state grinding, some can only be obtained by solid-state grinding. Failure to form co-crystals by grinding may be due to an inability to generate suitable co-crystal arrangements rather than due to the stability of the initial phases (Blagden et al., 2007).

Solid state grinding is not a new method for co-crystal formation – it was first reported in the late 19th century. By this method, co-crystals were prepared by co-grinding two parent compounds together by machine or by hand. Many co-crystal materials could be prepared by both solid state grinding and solution growth. In some report recently, solid state grinding is used to reduce the experiment burden of co-crystal screening (Friscic, 2006). Moreover, it could also be used to prepare novel pharmaceutical co-crystal materials which are not readily accessible by solution growth. Successful examples of co-crystals prepared by this method include the co-crystal of caffeine with monocarboxylic acids (Rodríguez-Hornedo, Nehm.2006), and the co-crystal by carboxamide-pyridine N-oxide heterosynthon (Reddy. 2006).

The other technique than that has been state above that can be used for co-crystallization is reaction co-crystallization and melt.

2.4 Solubility analysis method

Solubility analysis of sample can be done by using gravimetric analysis method and by using high performance liquid chromatography (HPLC) analysis. This is to compare to the different value of solubility of the sample between the two methods. In this research, just CBZ is been analyze by using HPLC.

2.4.1 High performance liquid chromatography (HPLC)

HPLC (as shown in Figure 2.2) is a technique most commonly used for the quantitation of drugs in pharmaceutical formulations. HPLC involves the simultaneous separation and quantitation of compounds in a sample matrix that has been introduced onto a chromatographic column, packed with a stationary phase. Separation is achieved by the use of a stationary phase and a solvent, termed the mobile phase, that is allowed to flow through the stationary phase at a set flow rate, for isocratic chromatography (Dumortier et al., 2001 and Meyer et al., 2002).

During analysis, the sample components partition to differing degrees between a stationary and mobile phase, based on their inherent physico-chemical properties (Meyer et al., 2002). The nature of the physico-chemical interaction between the mobile and stationary phase allows solute molecules to emerge from the column in individual component zones or bands, which are then monitored as a function of an appropriate detector response versus time.