SCREENING OF CARBAMAZEPINE-SACCHARIN (CBC-SAC) CO-CRYSTAL

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SCREENING OF CARBAMAZEPINE-SACCHARIN (CBZ-SAC) CO-CRYSTAL

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Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
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Dedicated to humanity.

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ABSTRACT

Co-crystallization is a method in formulation of drug products which is believed to improve drugs solubility, stability and dissolution rate while maintaining the biological functions of its chemical properties. The recent advances in this area have brought this research possible in order to produce pharmaceutical material by crystallization design of carbamazepine (CBZ) using saccharin (SAC) as its co-crystal. This research is conducted to examine formation of carbamazepine-saccharin (CBZ-SAC) co-crystal screening approaches that uses different solvent based crystallization technique in varies solvent system (ethyl acetate solvent and formic acid solvent). In this method, to prepare the co-crystal product CBZ-SAC four crystallization technique are used which are cooling crystallization, solvent crystallization, slurry and stirring in varies solvent of ethyl acetate and formic acid. Different mol ratio of CBZ and SAC are being tested, in order to study CBZ-SAC co-crystal formation. Physical characterization of the co-crystal is being characterized by x-ray powder diffraction (XPRD), differential scanning calorimetry (DSC), fourier transform infrared spectroscopic (FTIR) and optical microscopic. The XPRD analysis had confirmed that only CBZ-SAC co-crystal in ethyl acetate solvent were successfully formed while in formic acid solvent the crystal formed were only SAC. The XRPD pattern profile analysis shown that CBZ-SAC Form I and Form II was produced from different ratios and different methods and it have their own melting point based from the DSC analysis which are 170-172°C and173-177°C respectively. The morphology of the crystal are mostly plate like and needle like shape which indicate Form I and Form II respectively for the polymorphic characterisation. Since two type of polymorph were successfully produced, it is shown that solvent, methods and ratios plays an important role to CBZ-SAC co-crystal formation. Further study in screening is needed for co-crystal formation assessment since there were already many factors proven in affecting the polymorphic formation of the co-crystal such as different methods, solvent and mole ratio. Besides that varies solvent type should be implemented to investigate if the solvent yield CBZ-SAC co-crystal formation and its polymorph.

ABSTRAK

Penghabluran adalah satu kaedah dalam formulasi produk dadah yang dipercayai boleh meningkatkan kadar kelarutan, kestabilan dan pembubaran dadah tersebut disamping mengekalkan fungsi biologi sifat kimianya. Kemajuan terkini dalam bidang ini dapat membuka peluang bagi melaksanakan kajian yang lebih terperinci dalam menghasilkan bahan farmaseutikal yang reka bentuk meggunakan penghabluran bersama carbamazepine (CBZ) dan sakarin (SAC) sebagai crystal sokongan. Kajian ini dijalankan untuk mengkaji pembentukan crystal carbamazepine-sakarin (CBZ-SAC) menggunakan teknik penghabluran yang berbeza di dalam sistem pelarut yang dimanipulasi iaitu dengan pelarut asid etil asetat dan pelarut asid formik. Empat teknik berbeza di gunakan dalam menghasilkan kristal CBZ-SAC iaitu teknik penyejukan penghabluran, penghabluran pelarut, buburan dan kacau dalam larutan asid etil asetat and asid formik. Penggunaan nisbah mol CBZ dan SAC yang berbeza juga diuji, untuk mengkaji pembentukan kristal CBZ-SAC. Pencirian fizikal kristal CBZ-SAC boleh dilakukan mengunakan pembelauan serbuk x-ray (XPRD), pengimbasan pembezaan kalorimeter (DSC), jelmaan fourier spektroskopi inframerah (FTIR) dan mikroskopik optik. Analisis XPRD telah mengesahkan bahawa hanya kristal CBZ-SAC dalam pelatut asid etil asetat elah berjaya membentuk krystal yang di ingini manakala dalam pelarut asid formik kristal yang terbentuk hanyalah SAC. Analisis XRPD profil corak menunjukkan bahawa CBZ-SAC Jenis I dan Jenis II dihasilkan daripada nisbah yang berbeza dan kaedah peghaluran yang berbeza dan mereka mempunyai takat lebur yang tersendiri. Berdasarkan daripada analisis DSC takat lebur CBZ-SAC Jelis I dan Jenis II adalah 170-172 ° C and173-177 °. Morfologi kristal kebanyakannya morfologi kristal dalam bentuk plat dan jarum dan ini menunjukkan terhasilnya Jenis I dan Jenis II berdasarkan morfologi kristal tersebut kepada pencirian polimorfik. Apabila dua jenis polimorf telah berjaya dihasilkan, ia menunjukkan bahawa pelarut, kaedah penghabluran dan nisbah mol memainkan peranan yang penting dalam pembentukan CBZ-SAC bersama kristal. Kajian lebih lanjut dalam pemeriksaan ciri-ciri kristal diperlukan untuk menilaian pembentukan kristal kerana sudah terbukti terdapat banyak faktor yang mempengaruhi pembentukan polimorf iaitu seperti kaedah penghabluran yang berbeza, nisbah mol dan jenis pelarut yang digunakan. Selain itu, berbagai jenis pelarut juga harus di cuba untuk mengkaji jenis kristal yang terhasil dan polimorfnya.

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LIST OF SYMBOLS

℃	Celsius
D◦	Dose number
°	Degree
ρ	Density
>	Higher than
μ	Micro
%	Percentage
pKa	Index to express acidity of the week acid
θ	Theta

LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
BSC	Biopharmaceutics Classification System
CBZ	Carbamazepine
CBZ-SAC	Carbamazepine-Saccharin Co-crystal
CCF	Co-crystal Former
CSD	Cambridge Structural Database
DSC	Differential Scanning Calorimetric
EA	Ethyl Acetate
FRA	Formic Acid
FS	Freely soluble
FTIR	Fourier Transform Infrared Spectroscopy
GRAS	Generally Recognized As Safe
HPLC	High-Performance Liquid Chromatography
IR	Infrared Spectroscopy
NCT	Nicotinamide
NCT-SAC	Nicotinamide-Saccharin
RC	Reaction Co-crystallisation
PI	Practically insoluble
S	Soluble
SAC	Saccharin
SEM	Scanning Electron Microscopy
SPS	Sparingly soluble
SS	Slightly soluble
SSNMR	Solid State Nuclear Magnetic Resonance Spectroscopy

SXRD	Single Crystal X-ray Diffraction
TGA	Thermogravimetric Analysis
VS	Very soluble
XRPD	X-ray Powder Diffraction
VSS	Very slightly soluble

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Co-crystal development has an increasing interest in the pharmaceutical field as it plays an important class of pharmaceutical material as it enhances the solubility, dissolution and consequent bioavailability of poor water-soluble drugs by forming a crystal and a co-former with specific stoichiometry composition, (Thakuria et al., 2012). CBZ is an insoluble drugs that has a high dose requirement (>100mg/day) for therapeutic effect and possess multiple challenges for oral drug delivery, including a narrow therapeutic window, auto induction of metabolism and dissolution-limited bioavailability, (Hickey et al., 2007; Bertilson and Thomson, 1986; Meyer et al., 1992).

This co-crystallization technique is an approach that allows binding active pharmaceutical ingredient (API) with one or more components of co-crystal former (CCF) without breaking or making new covalent bond within one periodic crystalline lattice, (Sheikh et al., 2009; Gagniere et al., 2009). This will then preserve the biological function of the drugs while increasing its solubility performance. Co-crystal can be prepared by several methods such as solvent based method and solid based method. The solvent based methods involve slurry conversion via solvent evaporation, cooling crystallization and precipitation while the solid based methods involve dry grinding and solvent-assisted grinding (Pandrela, 2010).

It has been reported that co-crystal commonly characterize using x-ray powder diffraction (XRPD) as stated by Qiao (2011). Besides that, fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) also can be used.

1.2 Motivation

Carbamazepine (CBZ) is classified as a class II compound with low aqueous solubility. It is one of the water insoluble drug that face issues regarding poor solubility, bioavailability, stability and mechanical properties. Co-crystallization technique currently has been widely used in the pharmaceutical industry in order to improve the solubility of drugs since the drug often discarded during commercial production due to their low solubility. Co-crystallization of CBZ with SAC has been successfully produces and shows a promising result as solubility of CBZ-SAC increase greatly compare to the CBZ. However, it has been reported that CBZ-SAC co-crystal has two different polymorphic form and Form II is said to have improves the solubility of the drugs as stated by Porter et. al. (2008), while solubility data for Form I are not yet reported. Due to the polymorphic properties of the CBZ-SAC co-crystal it is important for us to know which screening method can produce the desired co-crystal which is CBZ-SAC Form II.

1.3 Problem Statement

It is true that the CBZ-SAC co-crystal have indeed successfully produced more polymorphs and this shows that CBZ-SAC have the higher potential to produce varies properties in term of solubility, bioavailability and stability. As it has been reported that CBZ-SAC co-crystal has polymorph issues, screening study needed to be implemented. Therefore, to come out from this problem CBZ-SAC co-crystal production is being tailor by manipulating the crystallization technique in term of method of crystallization, solvent used, and the mol ratios of the component. Many possible methods in crystallization is being review in order to know which method would produce higher probability of forming co-crystal of CBZ-SAC Form II. The method of producing CBZ with other CCF from other research will be used as a guideline and for further study.

1.4 Objective

Based on the research background and problem statement, the objective of this study is:

1) To study the formation of and Carbamazepine-Saccharin (CBZ-SAC) using varies solvent, solvent based crystallization method and concentration ratio [CBZ: SAC].

1.5 Scopes of Study

The scope of the research consists of:

1) Production of carbamazepine-saccharin (CBZ-SAC) co-crystal in ethyl acetate and formic acid solvent using cooling crystallization method, solvent evaporation method, slurry and stirring with different ratio of CBZ and SAC.
2) Characterizations of CBZ-SAC co-crystal using optical microscopic, powder x-ray diffraction (XRD), differential scanning calorimetry (DSC) and fourier transform infrared spectroscopic (FTIR).

1.6 Report Layout

This report contains five main chapter containing introduction, literature review, methodology, result and discussion, and conclusion. In the first chapter, the introduction briefly describes the background, objectives, and the structure of the proposed research. The second chapter, the literature review presented a brief and systematic overview of pharmaceutical co-crystals, definitions, basic theories of pharmaceutical co-crystals, recent progresses in pharmaceutical co-crystals and analysis that can be used to characterise co-crystal. The third chapter introduced all materials, analytical approaches, preparation and method development to conduct the experiment. In the fourth chapter, will includes results obtained from the experiment and discuss the obtained outcomes and the final chapter, the conclusion and recommendation concludes overall findings of this research and recommends a further improvement.

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CHAPTER 2

LITERATURE REVIEW

2.1 Chapter Overview

In this chapter, a review of pharmaceutical co-crystals was presented. First of all, co-crystal background was introduced, which will give the reader a general knowledge about co-crystal. The concept of the pharmaceutical co-crystals includes definitions, co-crystal basic formation and theory, physiochemical properties studies, recent progresses in pharmaceutical of co-crystals, and analysis that can be used to characterise co-crystal.

2.2 Co-crystal Background Review

2.2.1 Co-crystal Definition

Co-crystals can be defined in a number of ways. Within the academic literature, various parameters have been applied to the definition of co-crystal (Table 2.1); however, it can be agreed that that co-crystals are crystalline materials comprised of at least two different components commonly called multicomponent crystals which will be introduced in Section 2.2.2. One's idea as to what "component" is all about can be different in term of solid, liquid, or gas and/or neutral or ionic species, etc., and this is where the contrasting in definitions surface, (Schultheciss and Newman, 2009). Furthermore, the application of "pharmaceutical co-crystal" is usually used when an active pharmaceutical ingredient (API) is one of the molecules in the multicomponent crystal. Even though there are shortcomings with the definitions of co-crystal currently found in the literature, it is unnecessary to complicate the existing debate by generating yet another definition for what the co-crystal embodiment.

Table 2. 1: Literature definitions of co-crystal

Author	Definition of a co-crystal
Stahly, 2007.	"a molecular complex that contains two or more different molecules in the same crystal lattice"
Nangia and Bhogala, 2008.	"multi-component solid-state assemblies of two or more compounds held together by any type or combination of intermolecular interactions"
Childs and Hardcastle, 2007.	"crystalline material made up of two or more components, usually in a stoichiometric ratio, each component being an atom, ionic compound, or molecule"
Aakeroy and Salmon, 2005	"compounds constructed from discrete neutral molecular species...all solids containing ions, including complex transition metal ions, are excluded" "made from reactants that are solids at ambient conditions" "structurally homogeneous crystalline material that contains two or more neutral building blocks that are present in definite stoichiometric amounts"
Bond, 2007.	"synonym for multi-component molecular crystal"
Jones et. al., 2006.	"a crystalline complex of two or more neutral molecular constituents bound together in the crystal lattice through noncovalent interactions, often including hydrogen bonding"
Zaworotko et. al., 2006.	"are formed between a molecular or ionic API and a co-crystal former that is a solid under ambient conditions"
Almarsson and Zaworotko, 2004.	"Co-crystals are self-assembled at the molecular scale and can significantly expand the number of crystal forms."

In this Schultheciss and Newman review paper, the co-crystalline examples presented govern the following criteria:

1) Pharmaceutical co-crystal which formed by a neutral API and co-former through noncovalent interactions (example 1, Figure 2. 1), or salt co-crystal formed by ionic API and neutral conformer (example 2, Figure 2. 1) in a freely reversible interactions,
2) A co-former, which may or may not be pharmaceutically acceptable,
3) And at least one measured physicochemical property. An illustrated description of possible multicomponent systems, including co-crystals, salt co-crystals, and salts along with their respective hydrates and solvates are displayed in Figure 2.1.

When necessary for property or structural comparison, examples of pharmaceutical salts (example 3, Figure 2.1) will be introduced and discussed in Section 2.2.2.

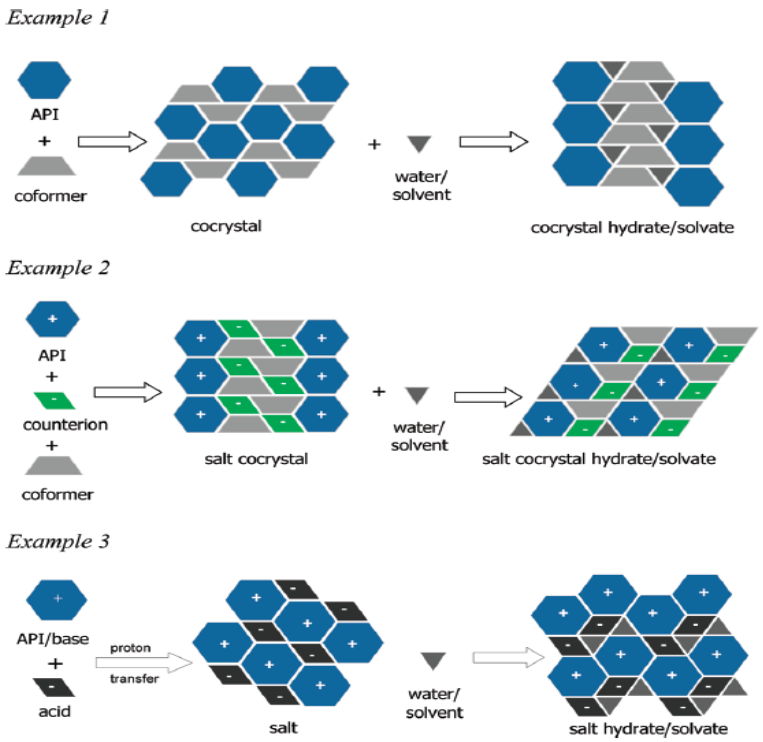

Figure 2. 1: Possible multicomponent system along with their respective solvate/hydrate forms

2.2.2 Understanding Co-crystal

Co-crystal occurs due to solid alteration from crystalline. It is known that crystalline materials acquire their physical properties from the arrangement of the molecule within the solid form, and altering the placement and/ or interactions between these molecules can have a direct impact on the properties of the particular solid, (Schultheciss and Newman, 2009). Currently, solid-state chemists call upon a variety of different strategies when trying to change the physical and chemical properties of APIs. APIs can exist in many different forms, including polymorph, hydrates, salts, solvates, co-crystal and amorphous solid as illustrated in Figures 2.2 and 2.4 due to different solid modification technique, (Morissette et. al., 2003). Every form have their own physiochemical properties that can greatly affect the bioavailability, manufacturability purification, solubility, stability and other performance of drugs, (Byrn et. at., 1999).

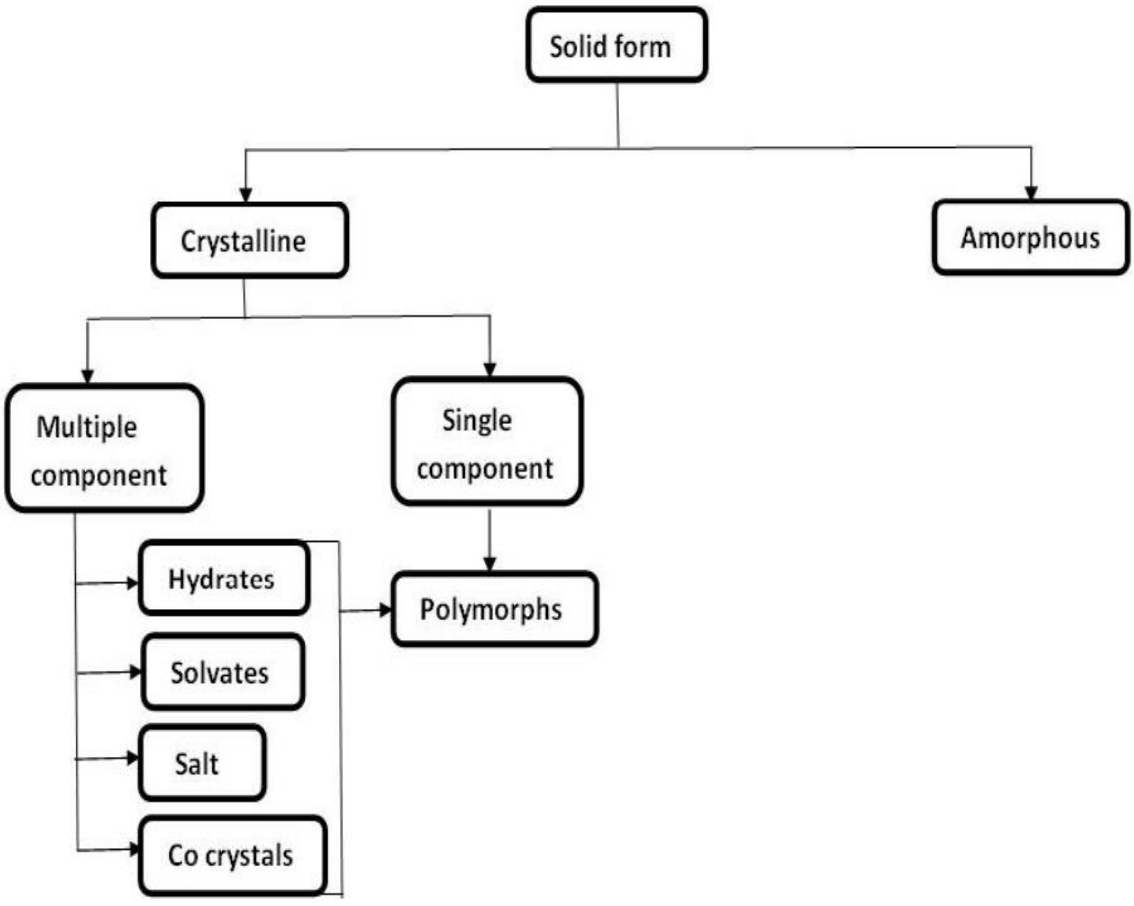

Figure 2. 2: Classification of API solid form based on molecular structure

As we can see from Figure 2.2 on classification of API solid form based on molecular structure, co-crystal is a part of a boarder family of multi-components crystal that includes solvates, clathrates, inclusion crystals, salts, and hydrates as shown in Figure 2.3. Salting involves acid–base reaction between API and acidic or basic substance, (Mundhe et. al., 2013). The important difference between co-crystals and solvates is the physical state of the isolated pure components. For example, at room temperature if one component is a liquid, the crystals are designated as solvates and if both components are solids, the crystals are designated as co-crystals, (Morissette et. al., 2013).

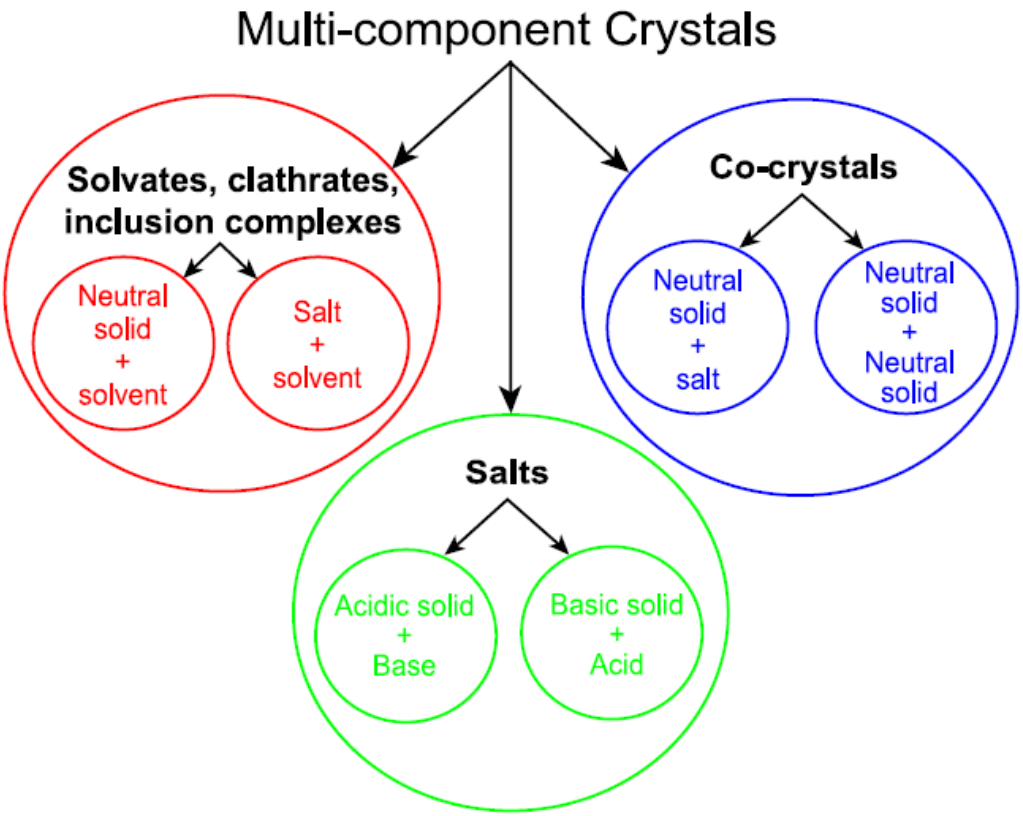

Figure 2. 3: Multi-components crystals

As we know APIs are usually regulated in the solid state as part of an approved dosage type in the forms of capsules, tablets and, etc., as long as the solids provide are practical and in compact format to store a drug, (Aakeroy et. al., 2007). Therefore, type of different crystals from multi-component crystal can be choosed depending on the most beneficial form in term of solibility, stability, and bioavailability.

A hydrate is typically a compound crystalline in which water molecules are chemically bound to another compound or an element. It is one of an effective approaches to improves properties as it has reported that 33% of molecules capable of forming hydrate, (Vippagunta et. al., 2001 ; Gillon et. al., 2003). Due to temperature and pressure cycling some hydrates changes into anhydrous crystalline form and result in physical property changes. Benefit of solvates in pharmaceutical field is very little because it tends to be more mobile and volatile due to the high vapor pressure. This will cause loss of solvent that will leads to amorphous compounds, which is chemically less stable and can crystallize into a less soluble forms, (Morissette et. al., 2003). Therefore, co-crystal are more convenient in pharmaceutical products than hydrates or solvates because they are most unlikely to evaporates from solid dosage form, making phase separation and other physical changes less likely to occur.

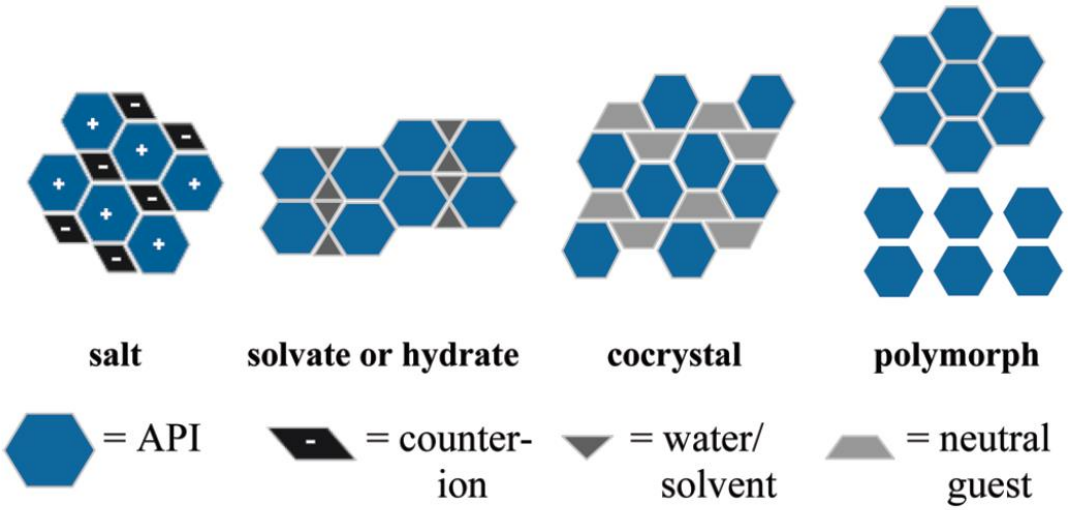

Figure 2. 4: Common solid state and their respected components

Polymorph is where the same molecules have two or more different arrangement and are showing different physicochemical properties, this phenomenon is termed as polymorphism, (McCrone, 1965). Even for a simple molecule it is not possible to predict number of polymorphs, (Motherwell et. al., 2002). Some compounds show more than ten crystal forms, (Rodrgueuez, 2004). The selection of solid modification technique is based on evaluation of solid and scope of screening method and as for the co-crystals the screening is fairly simple the main part is to find suitable pharmaceutical acceptable co-former. The identification of polymorphs is a major challenge for investigators as it requires high through put screening method, (Morissette et. al., 2004).

Polymorphism is a vital approach for designing co-crystals. Polymorphs can be reformed from unstable polymorphs to stable polymorph at particular temperature and they possess different melting point and solubility due to the difference in dissolution rate. A few examples of polymorphic co-crystals are nicotinamide-carbamazepine (NCT-CBZ) co-crystals and CBZ-SAC co-crystals, (Porter et. al., 2008).

Salt is one of another way to enhancement physical properties. Salt is used in the place of free acid and base in order to enhanced stability, solubility, and crystalline property of pharmaceutical compound. Salt is formed by transfer of proton from acid to base. As proton is involved the formation by measurement of pKa value can be predicted. Salts formation depends on the arrangement of hydrogen molecules in crystals. There are some marketed APIs which are result of solid modification by salt formation**.** Salts and co-crystals are two opposite aspects of multi-component system. The difference between co-crystals and salts is that drug and co-former are solids at ambient temperature and that the intermolecular interactions are non-ionic in nature, it can be seen through Figure 2.5 below, (Aakeroy et. al., 2005).

Solvates are a more or less loosely bonded complex formed between a solvent as it able to dissolve other substance and a dissolved species. Solvates development of API crystal is rare because they impart toxicity as most solvates are biologically toxic in nature. Basic difference between solvates and co-crystals is their physical form. If substance is liquid at room temperature it is considered as solvates, (Brahmankar and Jaiswal, 2005). Clathrates are a compound in which molecules of one component are physically trapped within the crystal structure of another.

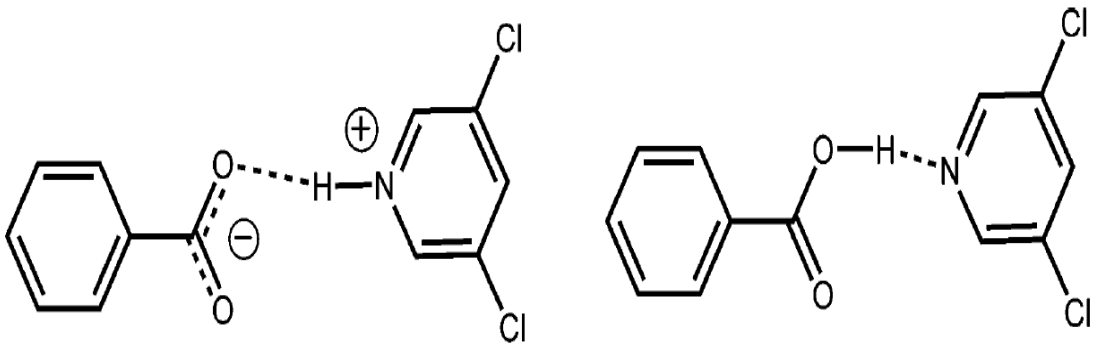

Figure 2. 5: Salt (left) and co-crystal (right)

2.2.3 Co-crystal Engineering

Pharmaceutical co-crystal can be designed by crystal engineering with an intention to boost the solid-state properties of an API without affecting its natural structure. Crystal engineering can be defined as an approach of the concepts of supramolecular chemistry to the solid state with particular emphasis upon the idea that crystalline solids are actual manifestations of self-assembly, (Qiao,2014). Co-crystals are constructed from intermolecular interactions such as van der Waals contact forces, π···π stacking interactions, and hydrogen bonding (Qiao,2014). The most common hydrogen bonds utilized in crystal engineering used in pharmaceutical co-crystals are shown in Fig. 2.5. Crystal engineering involves modification of the crystal packing by changing the intermolecular interactions of a solid material, (Miroshnyk et. al., 2009). The variety of structure observed provides hope that some forms will have superior performance in pharmaceutical dosage forms.

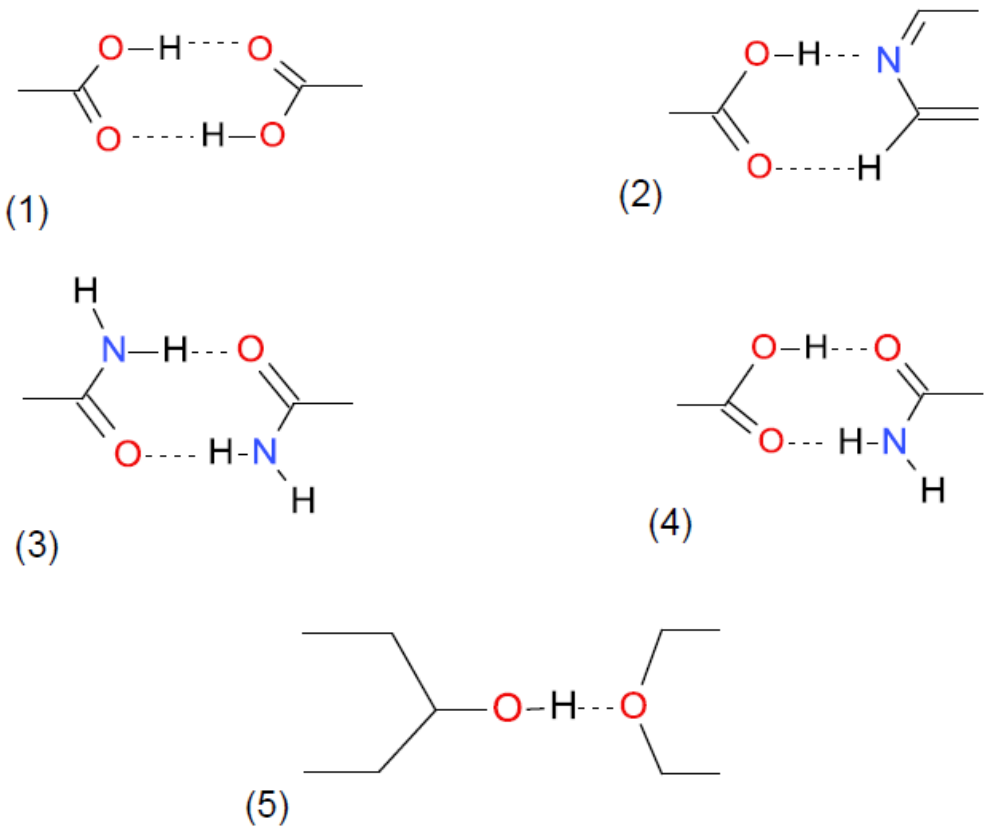

Figure 2. 6: Typical hydrogen bonds utilized in crystal engineering

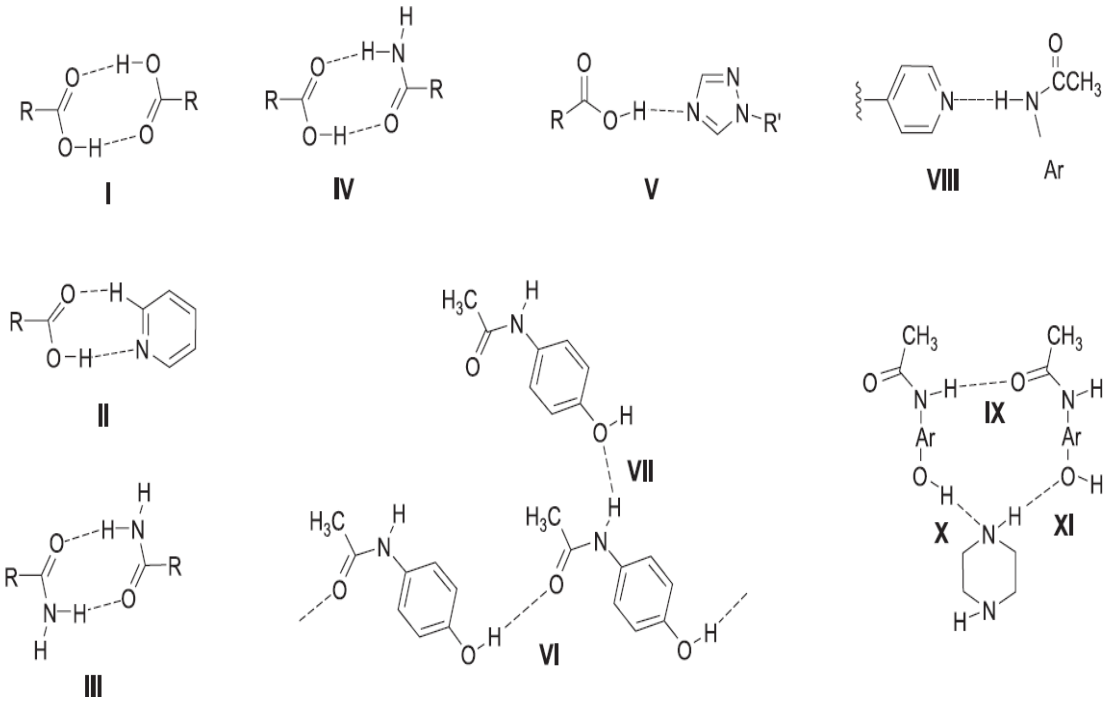

Figure 2. 7: Supramolecular synthons observed in co-crystal

Supramolecular processes in crystal growth nucleation and the growth of crystals have been widely reviewed in the literature, (Cabrerra and Vermilea, 1958). Nucleation is a molecular assembly process, where a critical number of molecules are needed to achieve the phase change from melt or solution into a crystal, (Blagden et. al., 2007).

2.2.4 Pharmaceutical Co-crystal Design Strategies

As pharmaceutical co-crystals have rapidly surfaced as a new class of API solids, much work has focused on exploring the crystal engineering and design strategies. Pharmaceutical co-crystals design is a multi-stage process, as schematically illustrated in Figure 2.5, (Miroshnyk et. al., 2009).

In order to get a desirable co-crystal product of an API, the first step is to study the structure of the target API molecule and find out the functional groups which can form intermolecular interaction with suitable co-formers. The next step is to choose a co-crystal co-former. The important demand for a co-former is to be pharmaceutically acceptable, for example, pharmaceutical excipients and compounds classified as generally recognized as safe (GRAS) for use as food additives. Selection of co-former is

the vital step for designing a co-crystal. During the design process, there are lots of worthwhile crystal empirical and theoretical guidance, such as Cambridge Structural Database (CSD), hydrogen bond theories, and many empirical conclusions. The next step is co-crystal screening, which is an experimental process to determine if a targeted API is able to co-crystallise with a particular co-former candidate. After a small scale screening exercise, proper co-formers could be selected to do scale up experiments. Various screening methods have been invented for co-crystal screening, such as solution method, computed crystal energy landscape method, and hot-stage thermal microscopy method. The goal of co-characterization is to examine the physical, chemical, and crystallographic properties of co-crystals. Usually, characterization includes the chemical structural conformation and crystallographic analysis of the newly formed supramolecular synthon, its thermal features, stability and solubility. Details of different co-crystal characterisation techniques will be given in section 2.2.6 on co-crystal characterisation techniques. The final step is the performance tests such as solubility test using HPLC.

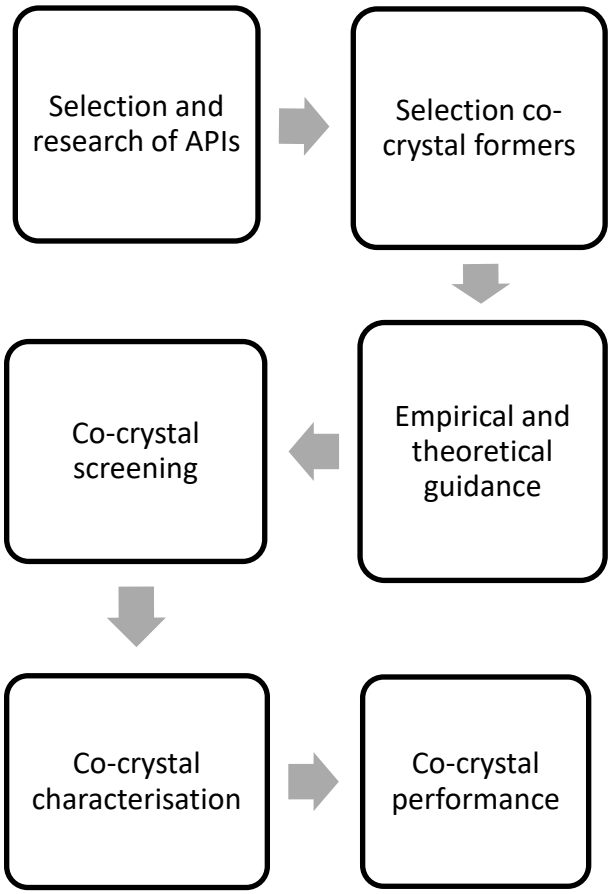

Figure 2. 8: Steps for co-crystal design and preparation

2.2.5 Co-crystal Formation Method

It have been reported that many strategies can influence and control the crystallization process to produce the solid form of interest, as can be seen in Figure 2.9. New processing methods continue to be invented to improve discovery and characterization of new forms. However, due to lack of fundamental understanding on the nucleation process and the specific factors that contribute to crystallization of diverse form of compound hardly any new discovery are found, (Morissette et. al., 2003). If the fundamental can be grasp, new experimental methodologies have the potential to have development.

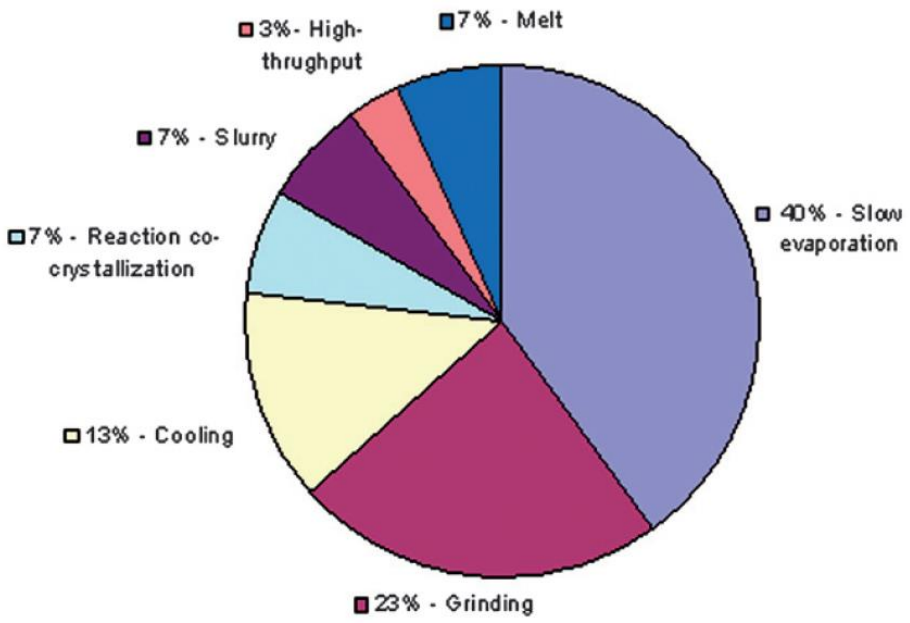

Figure 2. 9: Break down of techniques used for co-crystallization in open literature, (Sheikh et. al., 2003)

The most common method used for crystal formation is the use of suitable solution with suitable degree of supersaturation. There are several methods through which solution is supersaturated such as cooling, evaporation and incorporation of solubility lowering solvent or substance. The most frequent technique used for preparation of co-crystals is the evaporation. In executing the solvent evaporation method a series of events that follows are preparation of two or more suspensions by dissolution of stoichiometric amounts of materials in a solvent, mixing of suspensions and storage under suitable temperatures for co-crystallization. It is assume that in

evaporation process the solution of multiple molecules in suitable amounts are to undergo hydrogen bonding. Thermodynamic stability of molecules should always be considered to have optimal result during evaporation. However, the biggest drawback of evaporation process is its failure to comply in large scale preparation and co-crystallization through solution evaporation based crystal growth technique also did not help to give an optimal result. Some of a few more established techniques that currently used for co crystal formation are mechanical co-crystal synthesis and solvothermal co-crystal synthesis, (Mundhe et. al., 2013).

There are two different techniques for co-crystal formation via grinding. The first method is neat grinding, which is also called dry grinding, that consist of mixing the stoichiometric co-crystal components together and grinding them either manually, using a mortar and pestle, or mechanically, using a ball mill or a vibratory mill. To date many pharmaceutical co-crystals have been successfully synthesis by neat grinding, (Qiao, 2014). The second technique for co-crystal synthesis via grinding is that of liquid-assisted grinding also referred to as solvent-drop, wet co-grinding, in which minor amounts of appropriate solvent is added in which improve kinetics of co-crystal formation via grinding can be achieved by the addition of minor amounts of an appropriate solvent, (Qiao,2014).

Slurry crystallization is straightforward process which includes the addition of crystallization solvent in the components such as API along with its acceptable former. The selection of this process is primarily depends upon the physical stability of the crystallization solution to co-crystals and its solid former. The study on synthesis of co-crystals through Slurry crystallization was commenced in sixteen co-crystal system with optimum result. While preparation of co-crystals for Trimethoprim and sulfamethoxazole through slurry technique simple distilled water is used as solvent. Co-crystals of aspirin designed with 4, 4-Dipyridil as a co-former by using slurry crystallization method. However the yield obtained was not sufficient as compared with grinding method. The major disadvantage of this method is that it requires large amount of solvent.

In practice, solution co-crystallization is based on the following two strategies:

1) Use of solvents or solvent mixtures where the co-crystal congruently saturates and thus the components have alike solubility, or

2) Use of non-equivalent reactant concentrations in order to achieve the co-crystal stability region in non-congruently saturating solvents.

Strategy one is adopted when the two co-crystal components have alike solubility in solvent and solution co-crystallization with equimolar components will lead to the formation of the 1:1 co-crystal from solvent evaporation method. To date, many successful co-crystals were obtained by this technique. Strategy two is applied when co-crystal components have non-equivalent solubility, solution co-crystallization through evaporation of an equimolar solution may result in the formation of single component crystal or a mixture of individual component and co-crystal. The reaction co-crystallization (RC) approach has been selected for this situation. RC experiments are performed by adding the less soluble reactant to a saturated or close to saturated solution of the more soluble reactant and then the solution becomes supersaturated with respect to co-crystal.

Another solution method called cooling crystallization involves varying the temperature of the crystallization system, which has lateely attracted much more attention for potential of a large scale of co-crystal production. First, large amounts of reactants and solvent are mixed in a reactor typically a jacketed vessel, and then the system is heated to a higher temperature to make sure all solutes are totally dissolved in the solvent and is followed by a cooling down step. Co-crystals will precipitate when solution becomes supersaturated with respect to co-crystal as the temperature drops down.

In addition to this Rager and Hilfiker said that, utilizing solvent mixtures should lower as well the risk of solvate formation with any of the solvents in the mixture because the activity of each solvent is further decreased (Figure 2.10). Solvent mixtures provide an additional advantage for crystallization experiments in that they may also enhance the transformation kinetics. It has been shown experimentally for a few solvent combinations that the nucleation rate in solvent mixtures is generally higher than that in the pure solvent with the lowest nucleation rate. This can also be justified by theory as it has been argued that the nucleation is favored by a high solubility and preveted by

strong, directed interactions between solvent and solute. Mixed solvents will generally provide a larger number of less dominant interactions. Therefore, the activation energy for nucleation should be control more easily.

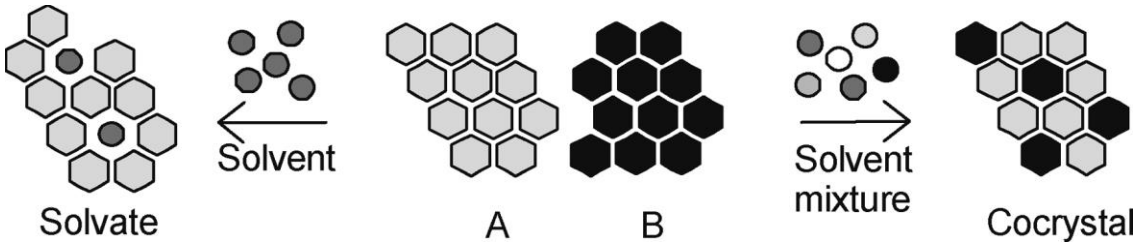

Figure 2. 10: Concept of Co-crystallization from Solvent Mixtures to prevent solvate formation

2.2.6 Co-crystal Characterization Technique

Co-crystal characterisation is an important part within co-crystal research. The basic physicochemical properties of co-crystals can usually be characterised by Powder X-ray Diffraction (PXRD), Single Crystal X-ray Diffraction (SXRD), Infrared spectroscopy (IR), Raman spectroscopy, Differential Scanning Calorimetry (DSC), Solid State Nuclear Magnetic Resonance Spectroscopy (SSNMR), Scanning Electron Microscopy (SEM), and Terahertz spectroscopy.

Table 2. 2: Characterization Technique

Characterization method	Determinant/Function	Disadvantage
SXRD	Determination of solid-state structure of co-crystals at an atomic level.	SXRD testing cannot always be produced.
PXRD	Verify the formation of co-crystals. (utilised more frequently)	Cannot distinguish solvates, hydrates or polymorphs from co-crystals, to make things worse, pharmaceutical co-crystals are prone to forming isostructural phases, (Miroshnyk et. al., 2009).

IR	Determining the chemical conformation of compounds. Can distinguishing co-crystals from salts when a carboxylic acid is involved in hydrogen bond formation, (Miroshnyk et. al., 2009).	-
Raman spectroscopy	Study vibrational, rotational and other low-frequency modes in a system, such as isostructural phase. Identify characteristic peaks of co-crystal products (Qiao,2014).	-
DSC	Thermal property testing of co-crystals (melting point data and additional thermal data). In addition to being a characterisation technique, DSC has recently been used as a screening tool for rapid co-crystal screening , (Miroshnyk et. al., 2009).	-
SSNMR	Characterisation of pharmaceutical co-crystals , (Miroshnyk et. al., 2009).	-
SEM	A type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. Determine the co-crystal micrograph and particle size in many examples, (Qiao, 2014)	-
Terahertz spectroscopy.	A versatile spectroscopic technique and an alternative to PXRD in the characterisation of molecular	-

	crystals distinguish between chiral and racemic hydrogen bonded co-crystals that are similar in molecular and supramolecular structure , (Miroshnyk et. al., 2009).	

It is important to stress that no single technique is completely adequate to characterize the properties of co-crystal. Integration of various characterization techniques could help clarified a better understanding of samples when analysing co-crystalline materials.

2.2.7 Type of Crystal Geometry

A crystal can be defined as a solid composed of atoms ions, or molecules which are arranged in an orderly and repetitive manner. It is highly organized types of matter. The atom, ions, or molecules are located in three-dimensional arrays or space lattices. The interatomic distances between these imaginary plane or spaces lattices in a crystal are measured by X-ray diffraction, are the angles between these planes. The pattern or arrangement of these space lattices is repeated in all direction (Geankoplis, 2014).

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

Cubic system. Three equals axes at right angles to each other.

Tetragonal system. Three axes at right angles to each other, one axis longer than the other two.

Orthorhombic system. Three axes at right angles to each other, all of different length.

Hexagonal system. Three equal axes in one plane at 600 to each other, and fourth axis at right angle to this plane and not necessarily at the same length.

Monoclinic system. Three unequal axes, two at right angles in plane and a third at some angle to this plane.

Triclinic system. Three unequal axes at unequal angles to each other and not 30,60, or 90.

Trigonal system. Three equal and equally inclined axes.

The relative development of different types of faces of a crystal may differ depending on the solute crystallizing.

2.2.8 Physicochemical Properties of Co-crystal

Physical and chemical properties of co-crystals are of great significance to the development of APIs. The overall motivation for investigating pharmaceutical co-crystals as other approach during drug development is the alteration of the physiochemical properties to improve the overall stability and efficacy of a dosage form, (Blagden et. al., 2008). The modification in molecular structure assemblies may cause changes in its physical properties. Thus, changes in physicochemical properties would be expected such as solubility, melting point, stability and bioavailability (Mundhe et. al., 2013).

Solubility is the property of a solid, liquid, or gaseous chemical substance called solute to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent. The solubility of a substance primarily depends on the solvent used as well as on temperature and pressure. The extent of solubility of a substance in a specific solvent is measured where adding more solute does not increase its concentration in the solution, (Savjani et.al, 2012). Table 2.4 show the type of solubility and Table 2.3 indicate that CBZ is practically insoluble. Therefore, by making CBZ to CBZ CCF it will change CBZ properties to more soluble.

Table 2. 3: Carbamazepine solubility, (Kasim et. al., 2003)

Drug	**Maximum dose strength (mg)**	**Solubility definition**	**Solubility (mg/mL)**	**Dose number (Do)**	**Therapeutic class**
Carbamazepine	200	PI	0.01	80	anticonvulsant, antiepileptic

Table 2. 4: Type of solubility, (Kasim et. al., 2003)

Descriptive term (solubility definition)	**Parts of solvent required for one part of solute**	**Solubility range (mg/mL)**	**Solubility assigned (mg/mL)**
Very soluble (VS)	<1	≥1000	1000
Freely soluble (FS)	from 1 to 10	100-1000	100
Soluble (S)	from 10 to 30	33-100	33
Sparingly soluble (SPS)	from 30 to 100	10-33	10
Slightly soluble (SS)	from 100 to 1000	1-10	1
Very slightly soluble (VSS)	from 1000 to 10000	0.1-1	0.1
Practically insoluble (PI)	10000	<0.1	0.01

Savjani et. al. said that solubility improvement techniques can be categorized in to physical modification, chemical modifications of the drug substance, and other techniques;

Physical Modifications.

Particle size reduction like micronization and nanosuspension, alteration of the crystal habit like polymorphs, amorphous form and co-crystallization, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

Chemical Modifications.

Change of ph, use of buffer, complexation, derivatization, and salt formation.

Miscellaneous Methods.

Supercritical fluid process uses of adjuvant like surfactant, solvency, hydro trophy, solubility, and novel excipients.

2.3 Carbamazepine (CBZ) Co-crystal Studies

2.3.1 CBZ Introduction

Figure 2. 11: Carbamazepine molecular structure

CBZ is an anti-epileptic drug with associated formulation issues including the existence of a variety of polymorphic forms (Florence et al., 2006; Grzesiak et al., 2003; Porter et al., 2008; Harris et al., 2005; Childs et al., 2009) and a tendency to form solvate or hydrate (Florence et al., 2006; Harris et al., 2005; Johnston et al., 2008). Due to its poor solubility which is 0.009 mg/mL (Kobayashi et al., 2000) it is usually required in high doses (>100 mg/day, D◦ > 40) for therapeutic effect. Oral administration of CBZ face multiple challenges, including low water solubility with high dosage required for therapeutic effect for example >100 mg/ day, dissolution-limited bioavailability and auto-induction for metabolism, (Shan and Zaworotko, 2008)

CBZ polymorph crystallizes as amide dimer, each of which tied up the polar amide functional groups through homosynthon III. Crystal structure shows that each dimer contains a peripheral H bond donor and acceptor pair that remain unused due to geometric constrain imposed by the drug molecule. Simple H-bond acceptors insert themselves to fills the voids between the adjacent pair of dimers. Multiple CCF having hydrogen bond acceptors likewise insert themselves into the void, (Morissette et. al., 2003). CBZ have at least four anhydrous polymorphic modifications and has been shown to form several solvates, including a stable dehydrate from aqueous solutions, (Bertilson et. al., 1986 ; Meyer et. al., 1992).

2.4 Carbamazepine-Saccharin (CBZ-SAC) Co-crystal

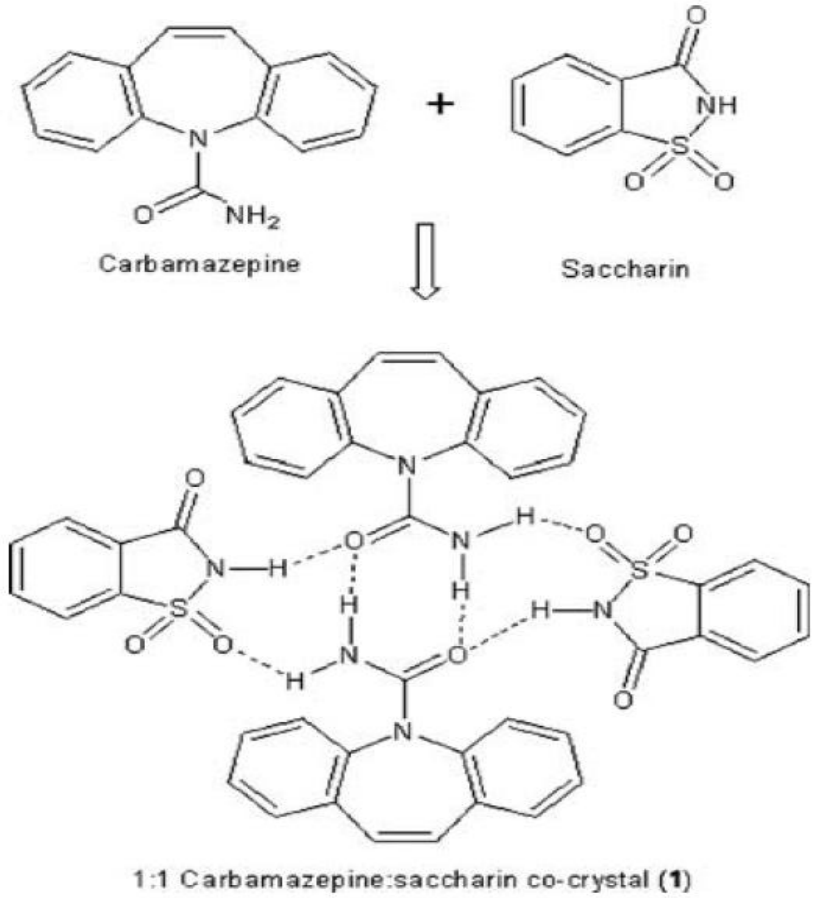

Figure 2. 12 : Carbamazepine: Saccharin (1:1) co-crystal components and schematics packing motif in methanol solution, (Hickey et.al.,2007)

In the solution with the presence of polymer heteronuclei two polymorph of CBZ-SAC co-crystal were grown and the resulting crystal from equimolar solution of CBZ-SAC in ethanol was analyse using crystal microscopy revealed that the two distinct crystal morphologies which are plates and needles suggest that the two form were indeed polymorphs and the CBZ-SAC II are more soluble than CBZ-SAC I, (Porter et. al., 2008). Analysis of the two CBZ-SAC co-crystal morphologies generate two distinct powder patterns that can be seen in the Table 2.5.

Table 2. 5: XPRD analysis data

CBZ-SAC I: plate like crystal	**CBZ-SAC II: needles like crystal**
$2\theta = 6.88°$	$2\theta = 4.80°$

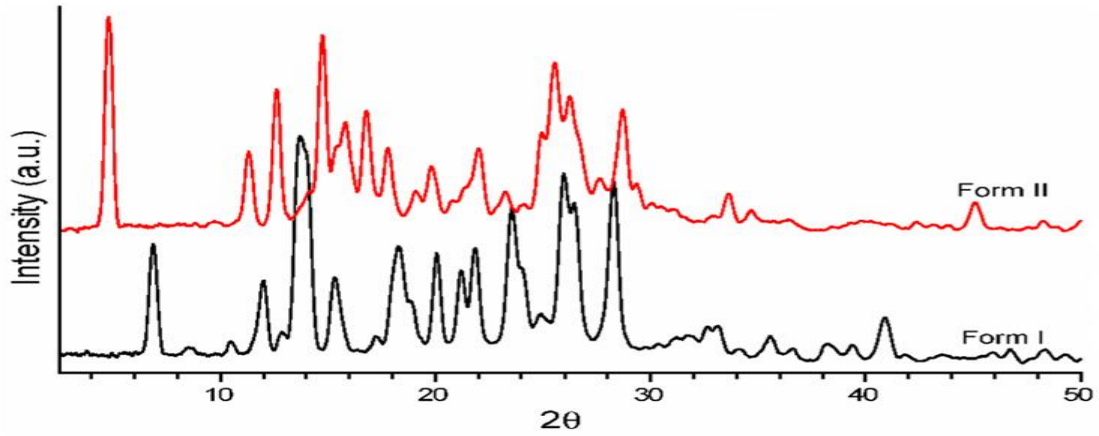

Figure 2. 13: PXRD pattern of CBZ-SAC I & CBZ-SAC II

Polymorphism of co-crystals will gain more attention as co-crystals continue to obtain more momentum during the development of new pharmaceuticals as co-crystal polymorphs offer additional options to alter properties, increase patent protection, and improve marketed formulations, (Schultheciss and Newman, 2009). So far only SAC and Nicotinamide (NCT) co-crystal of CBZ represent pharmaceutically are acceptable co-crystal, (Morissette et. al., 2003).

A studies carryout by Schultheciss and Newman on a dog study by comparing a 1:1 CBZ-SAC co-crystal with the marketed immediate release tablets of CBZ of Tegretol containing CBZ form III. This study conducted on the dogs noted that co-crystals can serve as a model for facing the physicochemical problems of a pharmaceutical compound in term of stability, solubility, dissolution, but will not solve issues of metabolism or pharmacology. Although the number of examples is limited, co-crystals can remarkably increase the bioavailability of poorly soluble compounds but there is not always a direct in vitro or in vivo correlation and even examples of dissociated co-crystals in in vitro studies can provide enhance performance in animal studies. It is reported that CBZ co-crystals were between 2 and 152 times greater than the solubility of the stable CBZ hydrate form, (Thakuria et. al., 2013).

2.5 Definition of basic pharmaceutical physical chemistry

Solution

A solution can be defined as a mixture of two or more components which are solvent and solute that will form a single phase, which is homogeneous down to the molecular level. Solvent influence the phase of the solution and usually compose the largest proportion of the system. Solutes are the components dispersed as molecules or ions throughout the solvent. Normally, the solutes are said to be dissolved in the solvent.

Solubility

The extent to which the dissolution proceeds under a given set of experimental conditions is referred to as the solubility of the solute in the solvent. Thus, the solubility of a substance is the amount of solute that passes into solution when equilibrium is established between the solution and excess substance which is the undissolved solute solid.

Type of solution

Solutions may be classified based on the physical phase of the solutes and solvent. All solutions studied in pharmaceutical research area have liquid solvents and the solutes are predominantly in solid phase.

Biopharmaceutics classification system

The biopharmaceutics classification system (BCS) is a system to distinguish drugs on the basis of their permeability and solubility. According to the BCS, drug substances are classified into four classes based on their aqueous solubility and permeability membrane properties, shown in Figure 2.14.

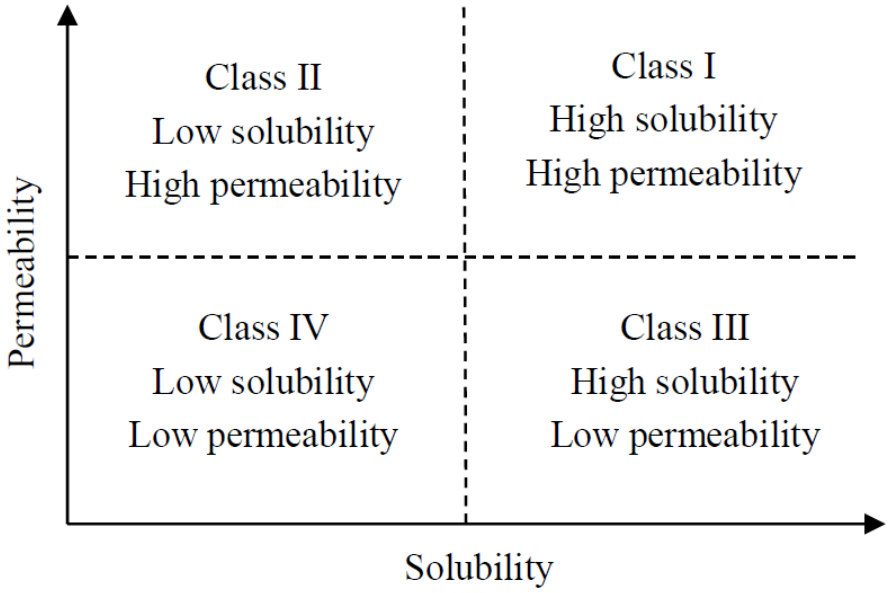

Figure 2. 14 : Biopharmaceutics classification system (BSC)

For BCS II and BCS IV drugs, the solubility enhancement can improve its oral bioavailability. Especially for BCS II drugs, which have low solubility and high permeability, solubility enhancement is a vital strategy to improve the oral bioavailability. The model drug studied in this research is, CBZ, which is categorized as a BCS II drug.

Polymorphism and transformation

In material sciences, polymorphism is a solid crystalline phenomenon of a given compound resulting from the ability of at least two different crystal structures of the molecules of that compound in the solid state. There are two types of polymorphism, monotropic and enantiotropic system. For a monotropic system, any transition between different polymorph is irreversible. For an enantiotropic system, it may be possible to convert reversibly between the two polymorphs on heating and cooling or through physical contact with a lower energy polymorph. For the model drug in this study CBZ, four polymorphs have been reported, CBZ form I to IV, (Qiao,, 2014). Among these polymorphs, CBZ form III is most stable form at room temperature and used in the marketed tablets products. CBZ III can transform to CBZ I through heating.

Polymorphism is crucial in drug and commercial product development in the pharmaceutical industry due to the properties of formulated pharmaceutical products such as bioavailability and stability are often directly related with the physicochemical and mechanical properties of the existing polymorphs in the formulation. In pharmaceutical industry, many drugs are under the regulatory approval for only a single crystal form or polymorph.

Active Ingredient

Any component of a drug product intended to equip pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of humans or other animals. Active ingredients include those components of the product that may undergo chemical change during the manufacture of the drug product and be present in the drug product in an altered form intended to equip the specified activity or effect.

Active Pharmaceutical Ingredient

Any substance or mixture of substances intended to be used in the formulation of a drug for medicinal product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to equip pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body.

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CHAPTER 3

METHODOLOGY

3.1 Chapter Overview

This chapter introduced all materials used for the experiment, analytical approaches used to obtain results, preparation needed to start the experiment and method used to conduct the experiment.

3.2 Materials

Carbamazepine (CBZ, purity 99%) and saccharin (SAC, purity 99.5%) were purchased from ECA International Corporation USA and Sigma Aldrich Chem. Company respectively. Whereas, ethyl acetate and formic acid both with purity exceeding 99% are purchased from Fisher company used as the solvent in the crystallization method in producing co-crystal.

Table 3. 1: Chemical details

Materials	Purity %	Molecular Weight (g/mol)	State of Material	Density, ρ (g/cm^3)	Application
Carbamazepine (CBZ)	99	236.27	Solid (Crystalline)	1.3	Co-crystal preparation
Saccharin (SAC)	99.5	183.1845	Solid (Crystalline)	0.828	Co-crystal preparation
Ethyl Acetate	99.8	88.11	Liquid	0.897 at 25ºC	As solvent for first parameter
Formic Acid	99.8	46.03	Liquid	1.22 at 25ºC	As solvent for second parameter

3.3 Experimental

3.3.1 Preparation of Carbamazepine-Saccharin (CBZ-SAC) Co-crystal

The experimental work was conducted using solution based approach via slurry crystallization and stirring crystallization methods. Carbamazepine and saccharin is weighed according to the different mol ratio as listed in Table I each with the interval of 0.25 started from 1.0 to 3.0 and an additional ratio of 0.50. The four different methods (Cooling crystallization, solvent evaporation, slurry and stirring) are used with two different solvent which are ethyl acetate and formic acid. The resulting solids from each different methods and solvents were separated using vacuum filter at room temperature. The solid produced from the experiment work were characterized using optical microscopic, x-ray powder diffraction (XRPD), differential scanning calorimetric (DSC), and fourier transform infrared spectroscopic (FTIR).

Table 3. 2: Experimental condition in preparing the solution in term of ratios

Saccharin (SAC)		**Carbamazepine (CBZ)**		**Mol Ratio SAC/CBZ**
Mass (g)	**Mol (mmol)**	**Mass (g)**	**Mol (mmol)**	
0.073	0.395	0.187	0.79	0.50
0.145	0.790	0.187	0.79	1.00
0.181	0.987	0.187	0.79	1.25
0.217	1.185	0.187	0.79	1.50
0.253	1.382	0.187	0.79	1.75
0.289	1.580	0.187	0.79	2.00
0.326	1.777	0.187	0.79	2.25
0.362	1.975	0.187	0.79	2.50
0.398	2.172	0.187	0.79	2.75
0.434	2.370	0.187	0.79	3.00

Molar mass, $_{SAC}$: 183.18 g/mol and molar mass, $_{CBZ}$: 236.264 g/mol.

CBZ mol was set as the constant variable with 0.79 mmol as referred from Zakiriah A. (2013). Using Equation 2.1 mass of CBZ can be obtained. Mol value of SAC can easily obtained from the set mol ratio and mass of SAC can be calculated from Equation 2.3.

$$mass_{CBZ} = molar\ mass_{CBZ}\ \frac{g}{mol} \times (0.79 \times 10^{-3}) mol_{CBZ} \quad \textbf{Equation 2.1}$$

$$mol_{SAC} = (0.79 \times 10^{-3}) mol_{CBZ} \times mol\ ratio \quad \textbf{Equation 2.2}$$

$$mass_{SAC} = molar\ mass_{SAC}\ \frac{g}{mol} \times mol_{SAC} \quad \textbf{Equation 2.3}$$

3.3.2 Cooling Crystallization Method

A. Ethyl Acetate Solvent

A mixture of CBZ with SAC with pre-defined mol ratios was mixed in 30 ml of ethyl acetate solvent in order for the mixture to be fully dissolved in a 50 ml conical flask and stirred for 72 hours at room temperature (~25ºC) in the orbital shaker with speed of 150 rpm. The speed is set to 150 rpm for all method as it is the ideal speed to produce higher production of co-crystal and also to standardize the method (Abd Rahim, n.d). It was important to make sure the solid mixed was dissolved in the solvent before starting the cooling down step so that the mixture of SAC and CBZ dissolve together to increase the chances to produce possible co-crystal. The temperature are then decrease by 5°C increment for 1 hour interval. At temperature 50-40°C co-crystal solid phase started to appear and the temperature was further lower to 25°C to drive additional precipitation. The resulting solid were filtered with a filter paper (0.2 μm), and let dried at room temperature and then analysed by optical microscopic, x-ray powder diffraction (XRPD), differential scanning calorimeter (DSC) and fourier transform infrared spectroscopic (FTIR). The experiment for cooling crystallization method in ethyl acetate solvent parameter was repeated for the other ratios.

B. Formic Acid Solvent

The experiment for cooling crystallization method for formic acid solvent parameter is conducted like the previous method with exchanging the solvent with only 10 ml formic acid solvent in order for the mixture to be fully dissolved.

3.3.3 Solvent Evaporation Method

A. Ethyl Acetate Solvent

A mixture of CBZ with SAC with pre-defined mol ratios was mixed in 40 ml of ethyl acetate solvent in a 50 ml conical flask and stirred at room temperature (~25ºC) in the orbital shaker with speed of 150 rpm. The speed is set to 150 rpm. It was important to make sure the solid mixed was dissolved for 1 hour in the solvent before proceeding to the next step so that the mixture of SAC and CBZ dissolve together and increase the chance of possible production of co-crystal. The dissolve mixture was then filtered using a syringe filter to eliminate impurities that will affect the formation of the co-crystal. The filtered mixtures are then located in to 50 ml conical flask covered with parafilm with small holes for the evaporation to occur. The samples are then left at room temperature for the solid to form. The duration for the solid to appear was around 20-25 days. The resulting solid were filtered with a filter paper (0.2 μm), and let dried at room temperature and then analysed by optical microscopic, x-ray powder diffraction (XRPD), differential scanning calorimeter (DSC) and fourier transform infrared spectroscopic (FTIR). The experiment for cooling crystallization method in ethyl acetate solvent parameter was repeated for the other ratios.

B. Formic Acid Solvent

The experiment for solvent evaporation method for formic acid solvent parameter is conducted like the previous method with exchanging the solvent with 30 ml formic acid solvent in order for the mixture to be fully dissolved. The duration for the solid to appear was roughly around 6 months.

3.3.4 Slurry Method

A. Ethyl Acetate Solvent

A mixture of CBZ with SAC with pre-defined mol ratios was slurried in 10 ml of ethyl acetate solvent in a 50 ml conical flask and stirred for 72 hours at room temperature (~25ºC) in the orbital shaker with speed of 150 rpm. The speed is set to 150 rpm as it is the ideal speed to produce higher production of co-crystal (Abd Rahim, n.d).

The resulting solid were filtered with a filter paper (0.2 μm), and let dried at room temperature and then analysed by optical microscopic, x-ray powder diffraction (XRPD), differential scanning calorimeter (DSC) and fourier transform infrared spectroscopic (FTIR). The experiment for slurry method in ethyl acetate solvent parameter was repeated for the other ratios.

B. Formic Acid Solvent

The experiment for slurry method for formic acid solvent parameter is conducted like the previous method with exchanging the solvent with formic acid solvent and changing the mass mixture of CBZ with SAC of each mol ratios amplified by 3 and slurried in 30 ml of formic acid solvent in a 50 ml conical flask. The reason that value of solvent and both mixture of CBZ and SAC were amplified by 3 is to increase production of solid produced so that there is enough product for the analysis.

3.3.5 Stirring Method

A. Ethyl Acetate Solvent

A mixture of CBZ with SAC with pre-defined mol ratios was mixed and stirred in 10 ml of ethyl acetate solvent in a 30 ml vial and stirred for 72 hours at room temperature (~25ºC) with speed of 150 rpm. The resulting solid were filtered with a filter paper (0.2 μm), dried at room temperature and analysed by optical microscopic, x-ray powder diffraction (XRPD), differential scanning calorimeter (DSC) and fourier transform infrared spectroscopic (FTIR). The experiment for slurry method in ethyl acetate solvent parameter was repeated for the other ratios.

B. Formic Acid Solvent

The experiment for stirring method for formic acid solvent parameter is conducted like the previous method with exchanging the solvent with formic acid solvent.

3.4 Analytical Instruments

The analysis being run to identify the character of the solid sample produced was optical microscopic, XRPD, DSC and FTIR. The purposed of the analysis is mention in Table 3.3 below.

Table 3. 3: Analysis used for characterization

Analytical Instruments	Purpose
Optical microscopic	Identify shape of the co-crystal
X-Ray powder diffraction (XRPD)	Characterized of co-crystal using its peak pattern profile
Differential scanning calorimetric (DSC)	Analyse thermal properties of the co-crystal melting point
Fourier Transform Infrared Spectroscopic (FTIR)	Identify chemical bonds in a molecule by producing an infrared absorption spectrum

3.4.1 Characterization Analysis

Optical microscopic (Olympus BX51) was used to identify the crystal shape with magnification of 5x and10x depending on the visibility of the crystal in order to observe the morphology of the co-crystal. Sometimes the dried samples were difficult to visualize as the crystal tend to agglomerate. The samples were quite fragile and easy to be broken up so careful precaution during collecting the sample was needed to minimize error.

The X-ray powder diffraction (XRPD) was used to identify the powder pattern of the sample. The phase composition of the powder was established by showing different peak profiles using RIGAKU (Miniflex II) diffractometer with Cu Kα radiation. The system was operated at 30 kV and 15 mA with the 2θ (angle) from 3° to 40°. The step size and step time were 0.01° and 1 second/step, respectively.

The differential scanning calorimetric (DSC) was used to analyse the melting point of co-crystal. Sample that has bigger size of crystal was grind using mortar in order to get a uniform thermal contact with the cubicle pan. Around 2 to 3 mg sample are used and weighted in standard aluminum pan and analyzed in DSC from 30°C to 300°C, at heating rate of 10°C/min.

Fourier transform infrared (FTIR) was used to identify chemical bonds in a molecule. The FTIR attached with diamond detector at the wave number from 4000-600 cm^{-2} using 32 scans per spectrum with resolution 4 cm^{-1} was used for the analysis.

3.5 References

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter discuss and explain the results obtained from the analysis using optical microscopic, XPRD, DSC and FTIR on characteristic of co-crystal produced from cooling crystallization, solvent evaporation, slurry and stirring method. The comparison of type and probability on higher success of co-crystal production among these method will also being discuss.

4.2 X-ray Powder Diffraction (XRPD)

XPRD pattern shown in Figure 4.1 is a peak profile for the starting materials used in this study which are CBZ and SAC. As mention by Sehic et. al. (2010) each component has its own unique peak pattern as it can be seen from the Figures 4.2 and 4.3. From there, by comparing the co-crystal pattern with the starting material pattern the presence of co-crystal can be identified.

All sample produced using formic acid solvent does not indicate the presence of CBZ-SAC co-crystal formation since there are no unique pattern can be observed. The pattern obtained from the XRPD analysis for formic acid solvent parameter of sample produce as can be seen from Figure 4.2 are similar with the pure component of SAC. This is probably due to the type of solvent used as it does not promote better dissolution and binding of CBZ and SAC leaving only SAC being precipitated.

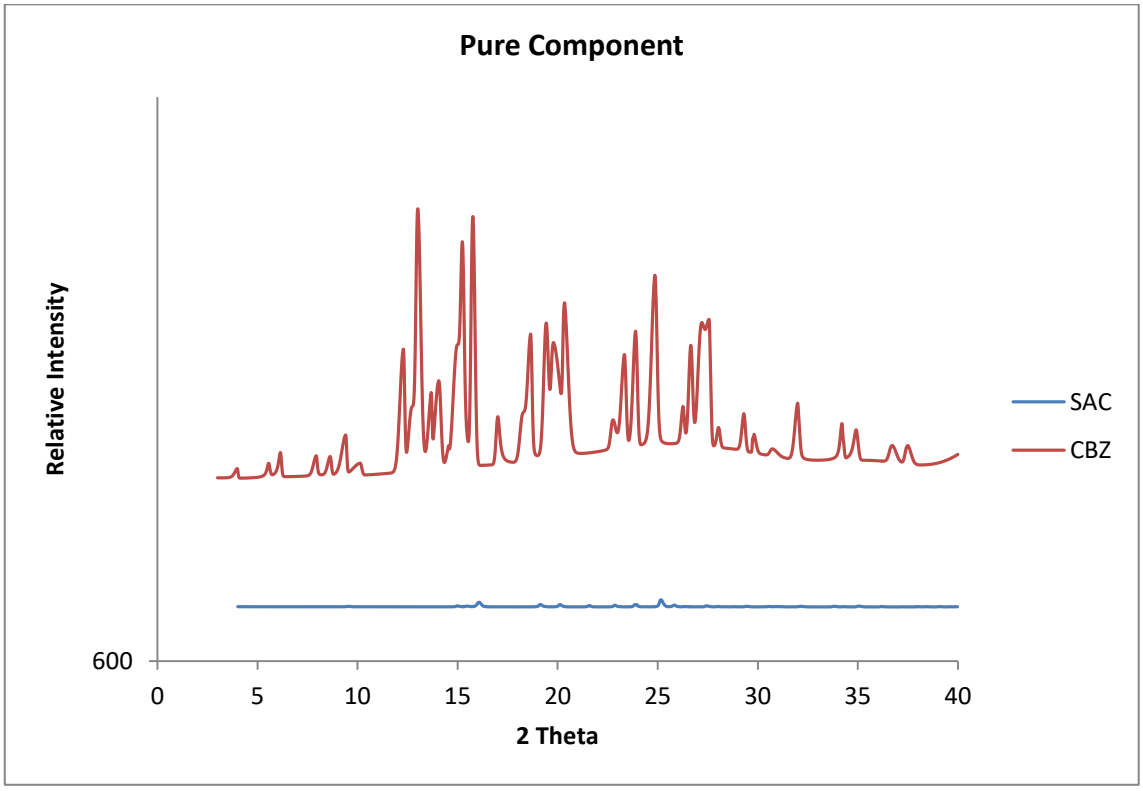


Figure 4. 1: XRPD for the pure component SAC and CBZ

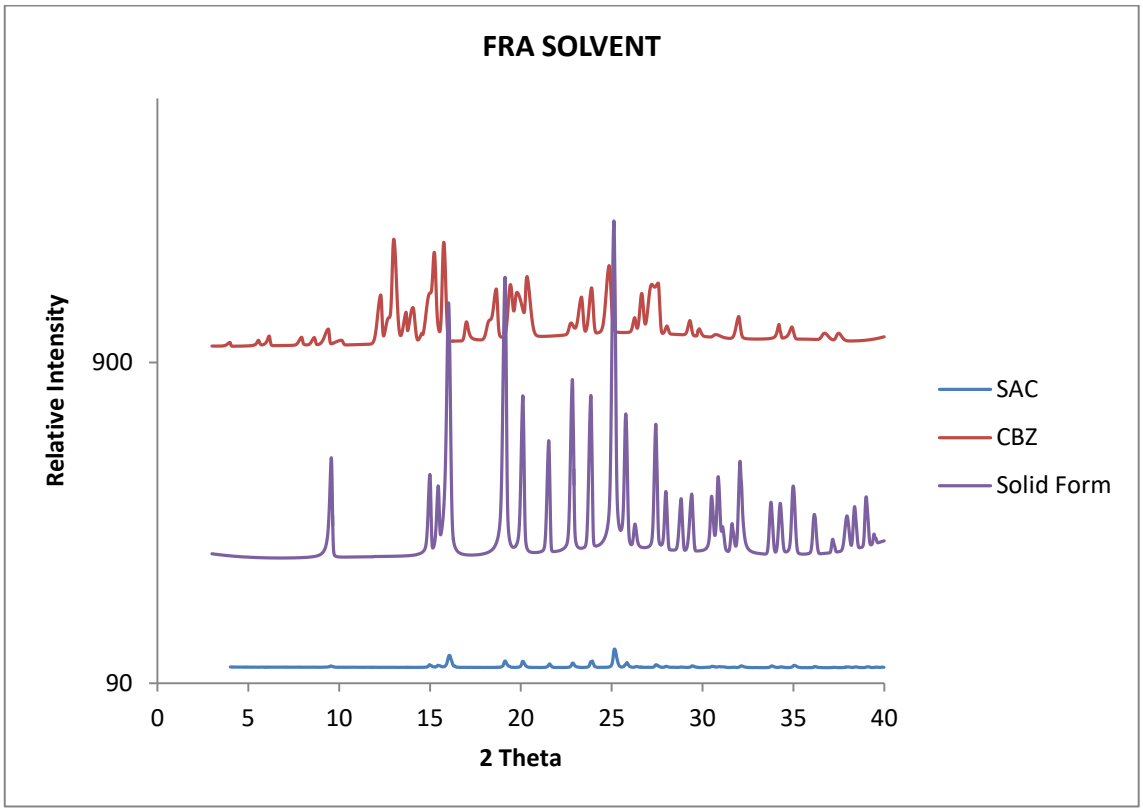


Figure 4. 2: XPRD for solid form in formic acid (FRA) solvent

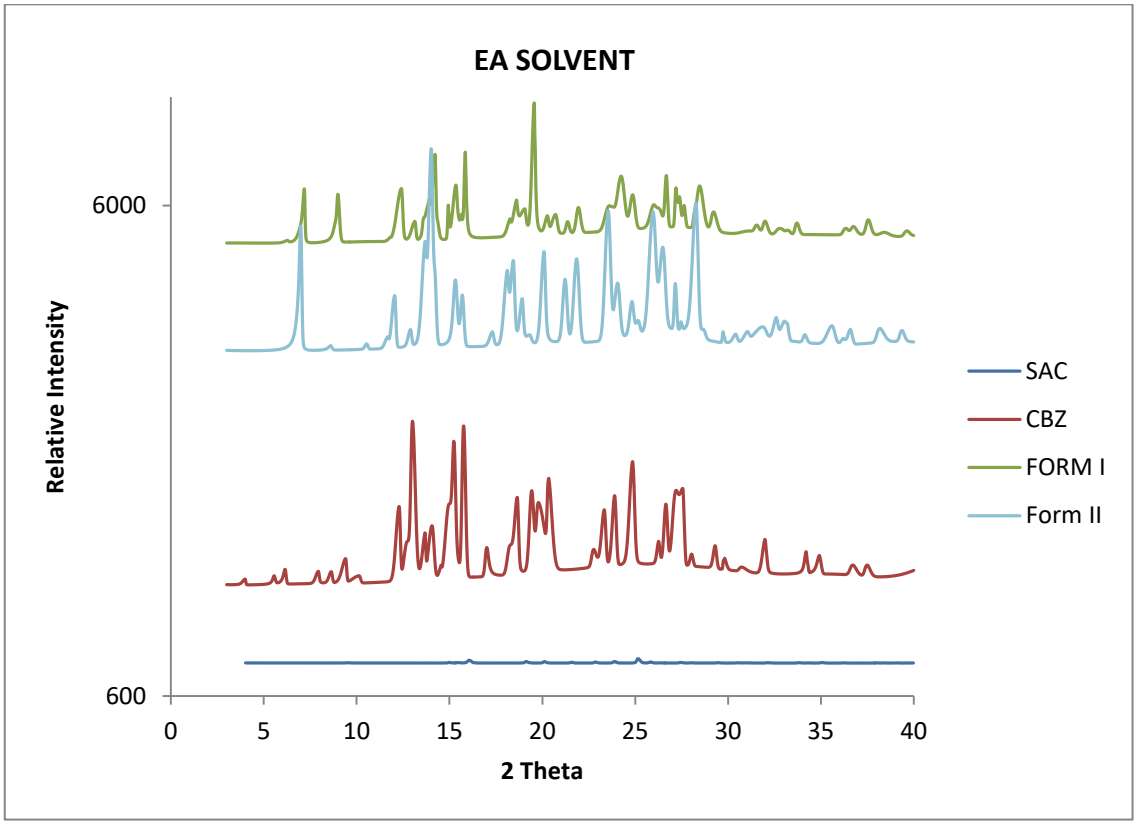


Figure 4. 3: XPRD for solid form in ethyl acetate (EA) solvent

From the XPRD analysis obtained from Figure 4.3 it was found that co-crystal has been successfully form in all of the method that use ethyl acetate as a solvent. The result pointed that CBZ-SAC has two polymorphic forms (i.e. Form I and Form II) depending on the type of method used. Evaporation method produces more CBZ-SAC Form I, while slurry, stirring and cooling method produces more of CBZ-SAC Form II. These finding are compatible with previous research saying that CBZ-SAC co-crystal are capable of producing two type of polymorph depending on method and solvent used. Besides that, as stated by Abd Rahim (2015), it is also agreeable that mole ratios also play a role in determining the polymorphic form of co-crystal.

Table 4.1 shows the summary of co-crystal formed based from solvent, method and mol ratio used. It can be seen that Form II was able to be form for all four method in ethyl acetate solvent while Form I only produced in slurry, stirring and evaporation method. Even though Form I was produced it also contain Form II which will be further discussed in Section 4.3 related to Figure 4.6 since the XRPD pattern of Form I does not contain profile pattern of Form II.

Table 4. 1: Summary result for co-crystal formation

Solvent	Screening Method	Mol ratio	CBZ-SAC Form I	CBZ-SAC Form II
Ethyl Acetate (EA)	Slurry	0.50	✓	✓
		1.00		✓
		1.25		✓
		1.50		✓
		1.75		✓
		2.00		✓
		2.25		✓
		2.50		✓
		2.75		✓
		3.00		✓
	Stirring	0.50	✓	✓
		1.00	✓	✓
		1.25		✓
		1.50		✓
		1.75		✓
		2.00		✓
		2.25		✓
		2.50		✓
		2.75		✓
		3.00		✓
	Evaporation	0.50	✓	✓
		1.00		✓
		1.25	✓	✓
		1.50		✓
		1.75	✓	✓
		2.00	✓	✓
		2.25	✓	✓
		2.50	✓	✓
		2.75	✓	✓
		3.00	✓	✓
	Cooling	0.50		✓
		1.00		✓
		1.50		✓
		2.25		
		2.50		✓
		3.00		✓

4.3 Differential Scanning Calorimetry (DSC)

Thermal properties of CBZ, SAC and its co-crystal were obtained from analysis of DSC shown in Figures 4.4 and 4.5. It can be seen that the melting point CBZ-SAC falls between the melting point of CBZ and SAC. The melting temperature of pure component obtained using DSC analysis is extracted from Figures 4.4 and 4.5 and tabulated in Table 4.2. The measure of melting point for CBZ and SAC obtained are in good agreement with the value reported by Padrela et. al., (2010) and Zakariah A., (2013), which are between 190 – 191°C and 227 - 229°C respectively.

Table 4. 2: DSC analysis result

Components	Melting Point (°C)
CBZ	190.43
SAC	227.53
CBZ-SAC Form I	170 - 172
CBZ-SAC Form II	173 - 177

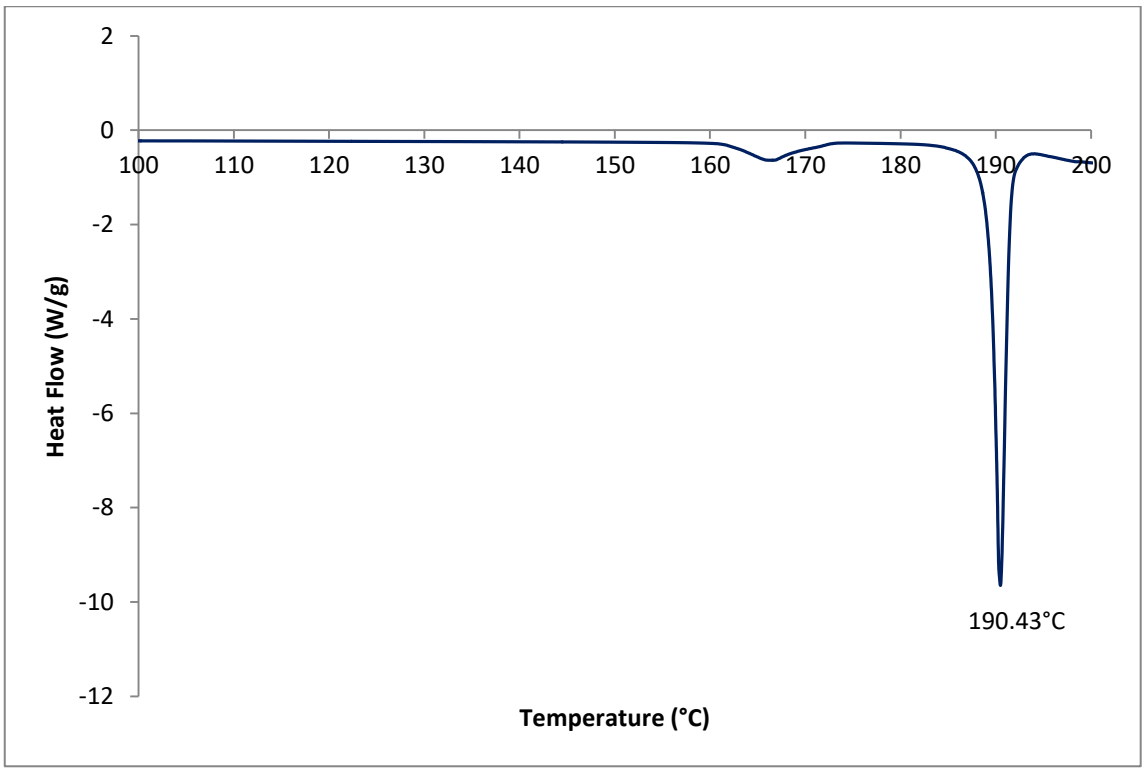


Figure 4. 4: DSC analysis for pure component of CBZ

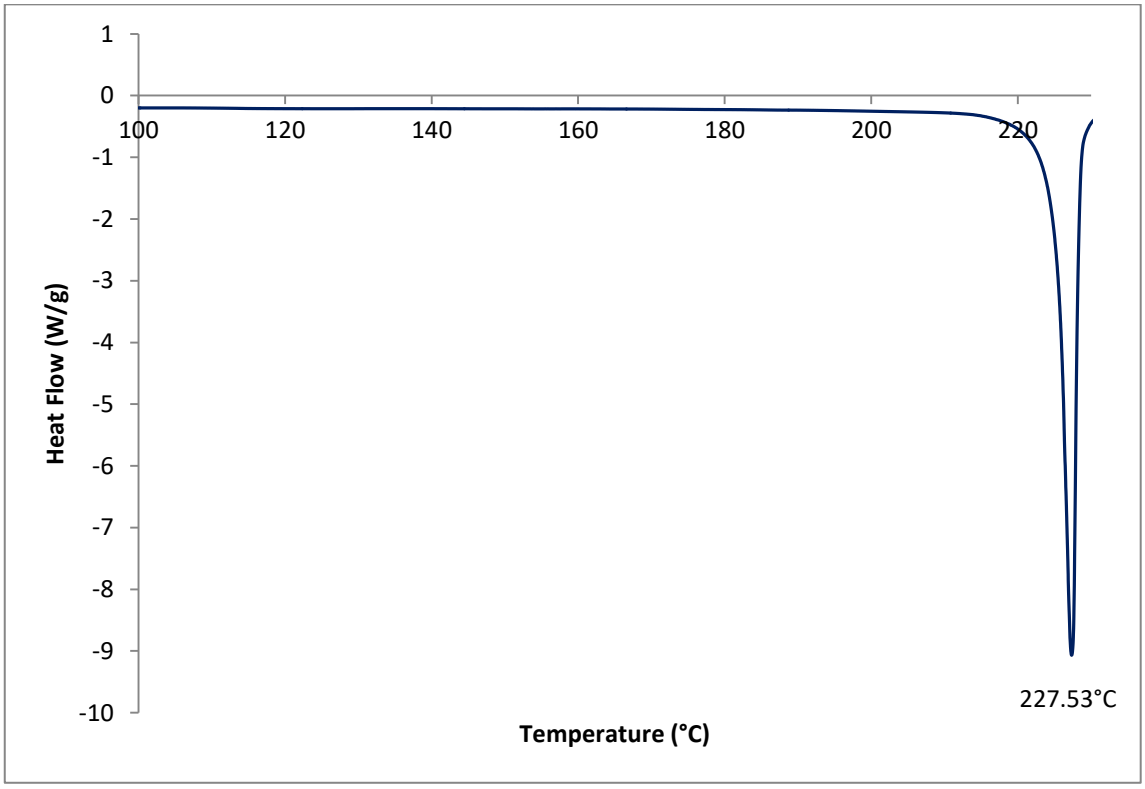


Figure 4. 5: DSC analysis for pure component of SAC

From Figure 4.6 polymorphic transformation of CBZ from Form I to Form II best describe the thermal event where polymorphic transformations take place at 170 - 172°C and new phase melted at 174-175°C which is probably Form II. The DSC analysis was able to detect the Form II and Form I together compare to the XRPD analysis probably due to the method used and different data collection view point for both analyses. This proved that DSC plays an important role in co-crystal characterization as well as polymorphic characterization, since different polymorph has different melting point. Form I has a melting point in the range of 170-172°C, while Form II at 173-177°C as can be seen on Figures 4.6 and 4.7. The increasing melting point of the two forms possibly because of the packing nature of the crystal in CBZ-SAC co-crystal (Sevukarajan et. al., 2011).

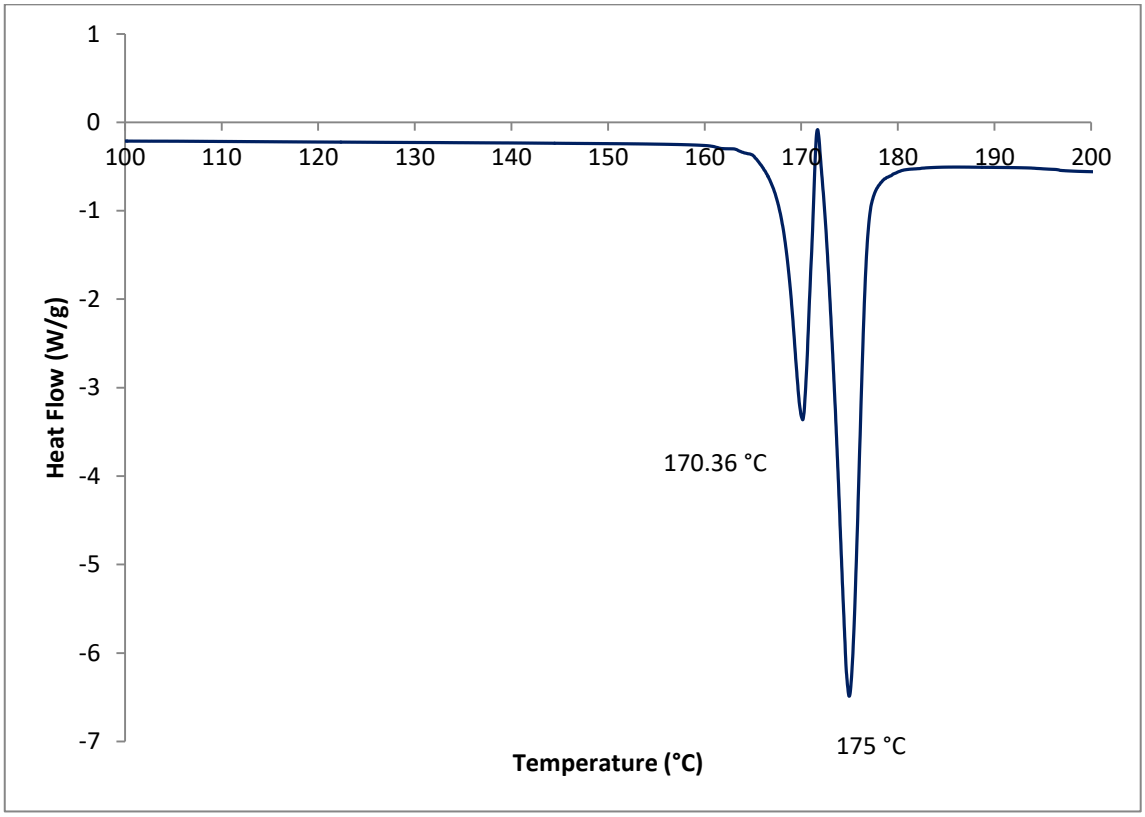


Figure 4. 6: DSC analysis for CBZ-SAC Form I co-crystal

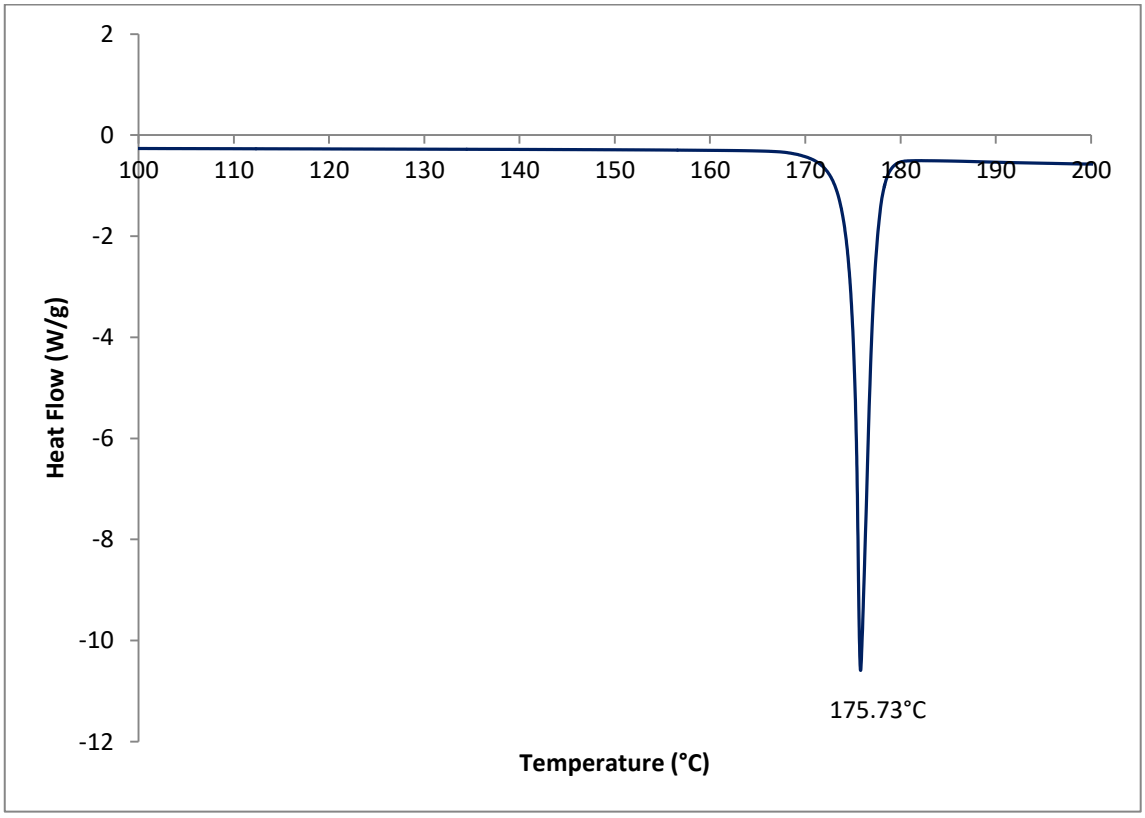


Figure 4. 7: DSC analysis for CBZ-SAC Form II co-crystal

4.4 Fourier Transform Infrared (FTIR)

FTIR was used for evaluating the interaction occurs between CBZ and SAC in the co-crystal. The FTIR spectrum of CBZ in Figure 4.8 reveals the characteristic stretching N-H bond form NH_2 at 3463.9 cm^{-1}, aromatic C-H stretching at 3156.09 cm^{-1}, C=C ring stretching at 1604.02 and 1593.37 cm^{-1}, and C=O stretching band at 1675.45 cm^{-1} which comply with Child and Rodriguez (2008).

The FTIR spectrum of SAC I Figure 4.9 reveals the N-H bond at 3099 cm^{-1}, C=O stretch at 1716.11 cm^{-1} and $–SO_2$ stretch at 1332.92 and 1174.82 cm^{-1}which comply with Jayasankar (2006).

Analysis of CBZ-SAC Form I and Form II by FTIR in Figures 4.10 and 4.11 reveals a drastic change in energy of the vibrational bands for N-H stretching modes in the 3500-3300 cm^{-1} region. The two N-H stretches of Form I are found at 3496.57 and 3435.12 cm^{-1} while the N-H vibrational bands of Form II are found at 3496.51 and 3350 cm^{-1}. Assuming that the N-H vibrational bands of CBZ are always higher in energy than the N-H vibrational bands of SAC (Jayasankar, 2006), these shifts represent a significant decrease in energy in of these modes in Form I. This is consistent with crystal structure of form II in which the measured hydrogen bond distance is significantly shorter than those measured in Form I. The lower free energy of CBZ-SAC Form I indicates that polymorph II is more soluble than Form I (Porter et. al.,2008).

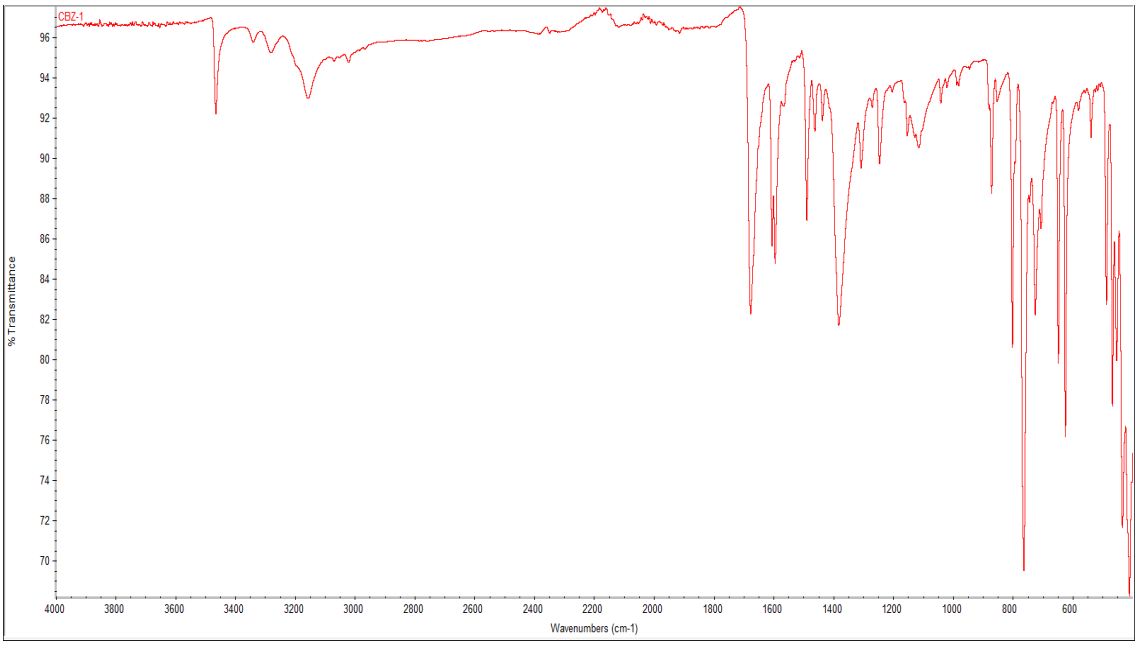


Figure 4. 8: FTIR for pure component CBZ

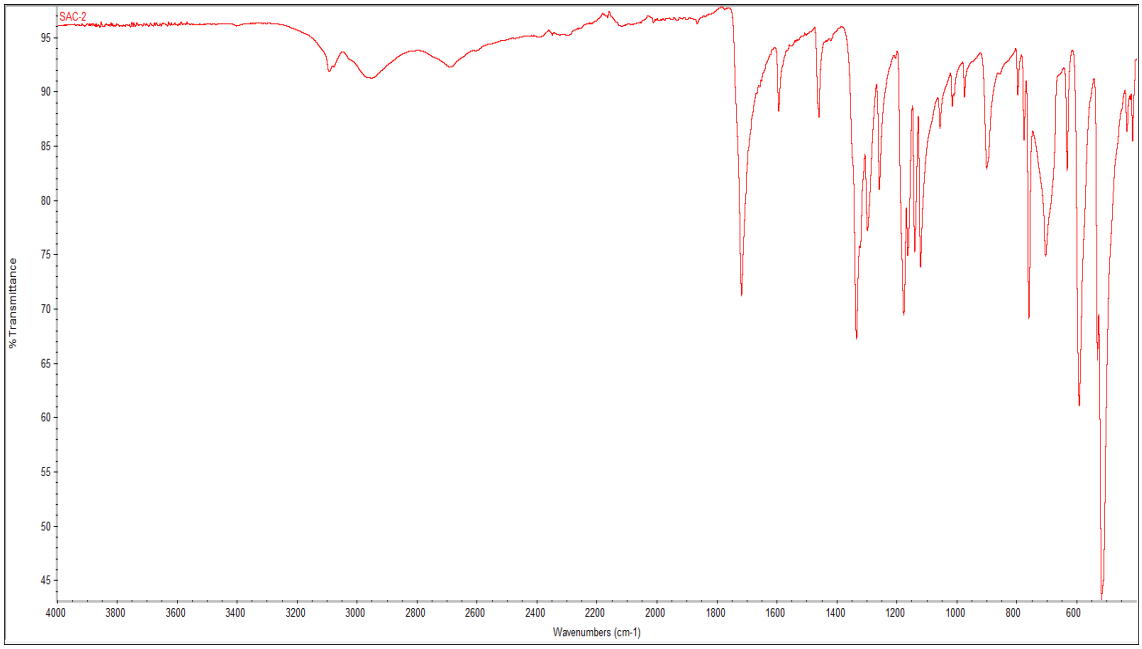


Figure 4. 9: FTIR for pure component SAC

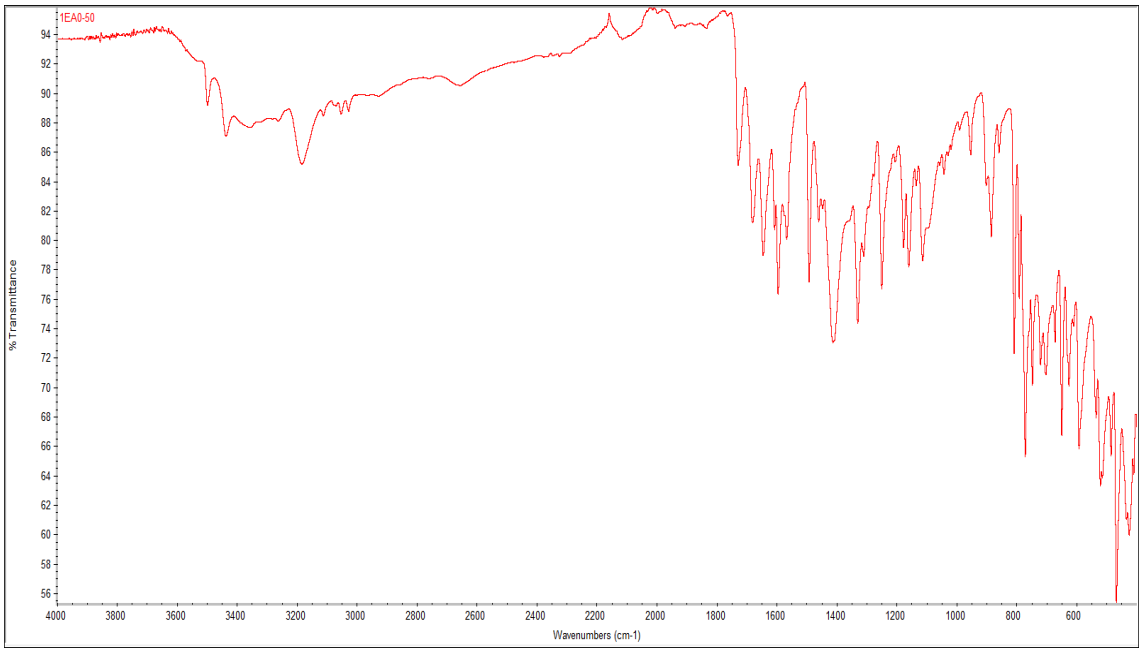


Figure 4. 10: FTIR for CBZ-SAC Form I

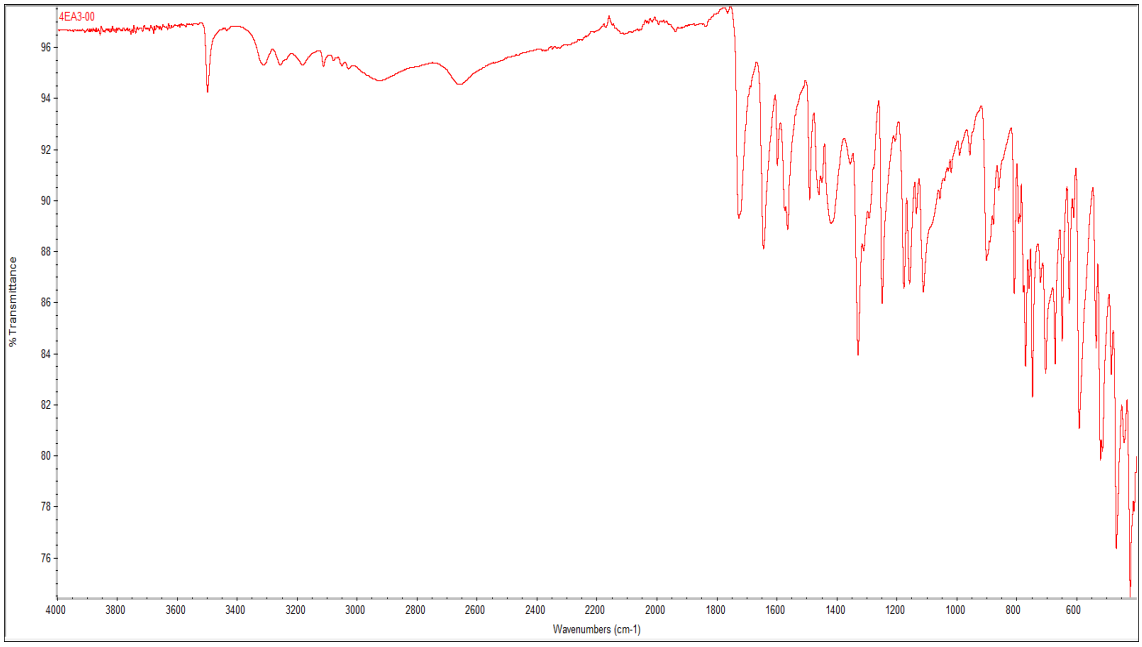


Figure 4. 11: FTIR for CBZ-SAC Form II

4.5 Optical Microscopic

The morphology of the product crystals obtained from solids is summarised in Table 4.2. The data show that the most successfully formed co-crystal is in plate like from. Most solvent evaporation method manage to produce needle like morphology except for 0.50 and 2.25 ratios in ethyl acetate solvent which is in plate like morphology and almost all ratios in formic acid solvent produce needle like morphology except for ratio 2.25, as the morphology is hard to distinguish due to agglomeration. For cooling crystallization all of the crystal produced are in plate like and for slurry and stirring method the morphology of most of the crystal are also in plate like form but due to agglomeration most of morphology of the crystal cannot be identified especially for the needle like morphology that is supposed to be observe in stirring and slurry method for ratio 0.50, and 0.50 and 1.00 respectively.

The crystal prepared in stagnant solutions (ie: solvent evaporation) tended to yield larger crystal making room for the nucleation growth not to be interrupted by agitation from shaking and stirring motion. From Figures 4.4 and 4.5 it can be seen that the size of crystal for slurry method are bigger than the stirring method by 47% to 60% larger in size. These were probably due to the more intense agitation in the stirring method than the slurry method as supported by Abd Rahim (2013).

Figure 4. 12: Stirring method morphology

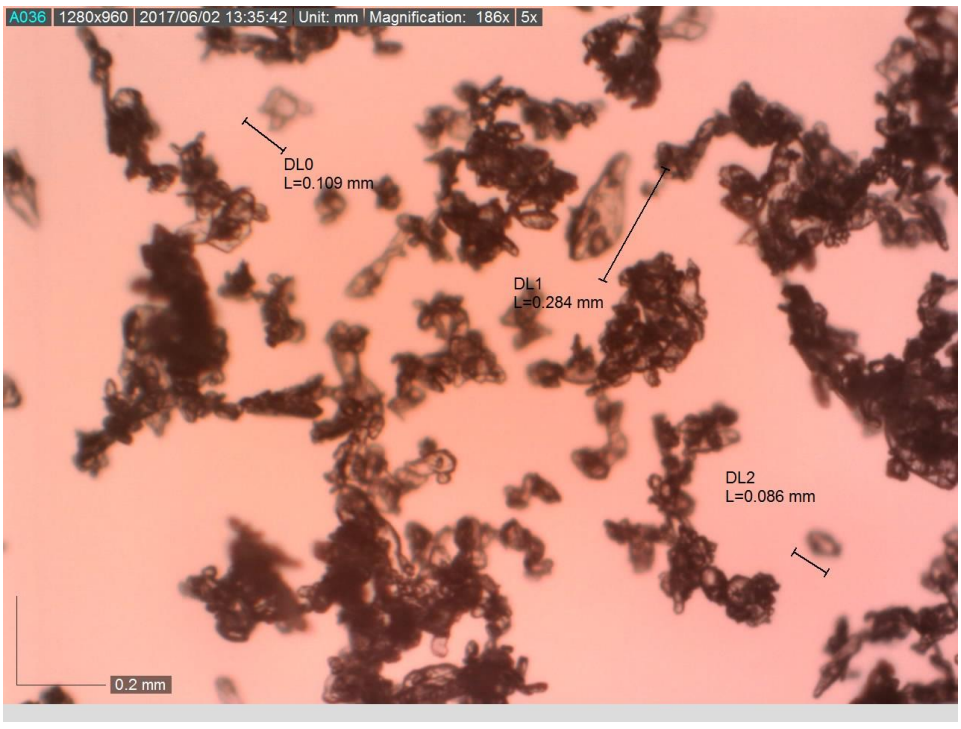
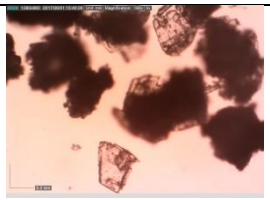
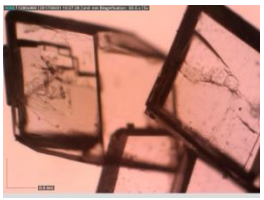
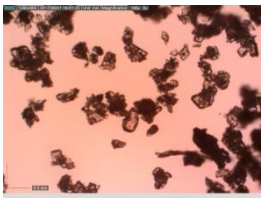
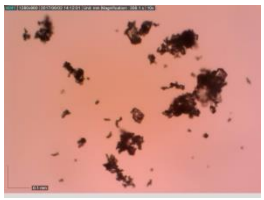
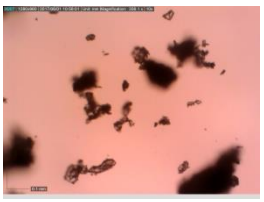
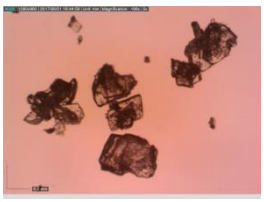
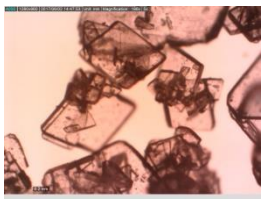
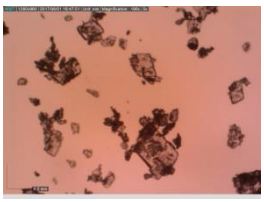
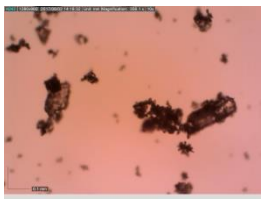
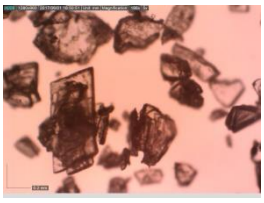
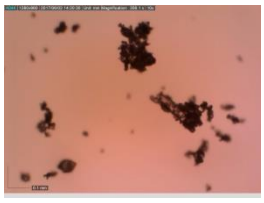
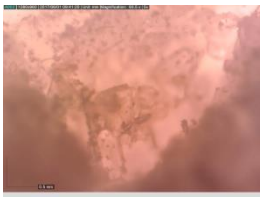
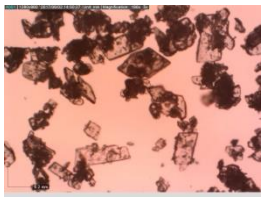
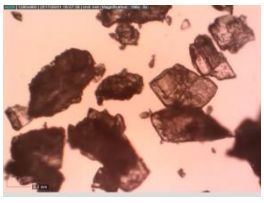
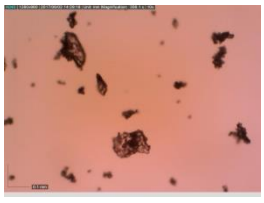
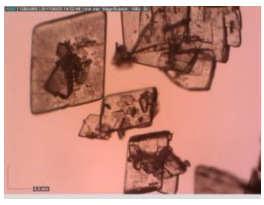
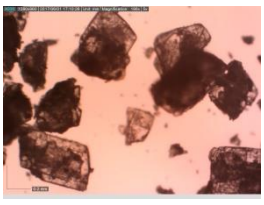
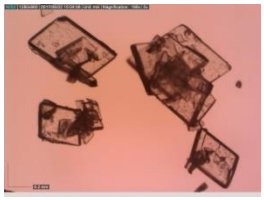
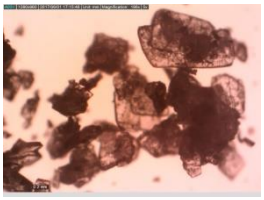
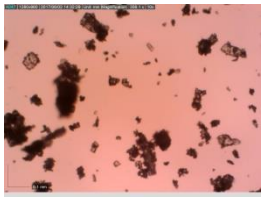
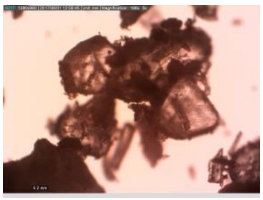
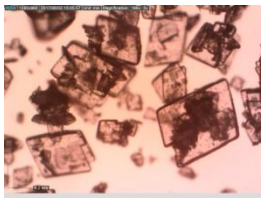
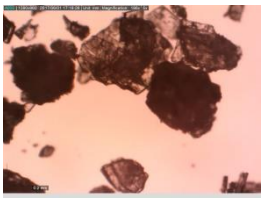
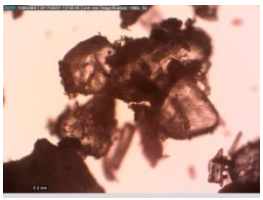
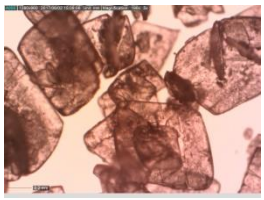
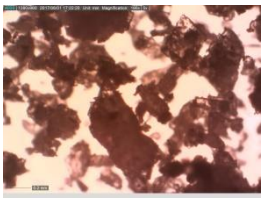
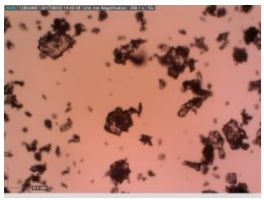


Figure 4. 13: Slurry method morphology

Table 4. 3: Optical microscopic analysis of co-crystal

Ratio	Methods			
	Solvent Evaporation	**Cooling Crystallization**	**Slurry**	**Stirring**
	Ethyl Acetate Solvent			
0.5		NA		
1.0		NA		
1.25		NA		
1.50				
1.75		NA		
2.00				
2.25				

2.50				
2.75				
3.00				
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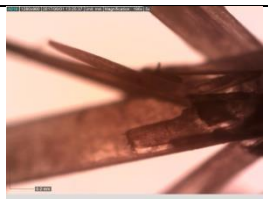
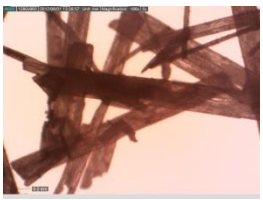
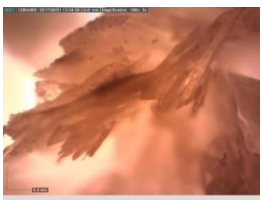
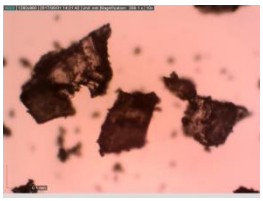
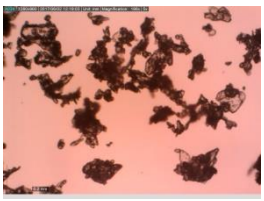
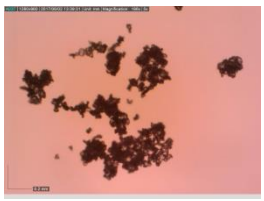
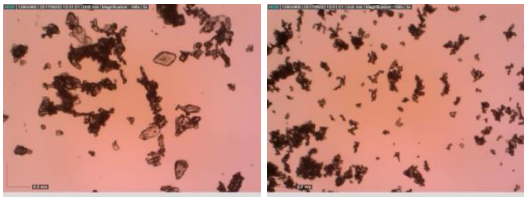
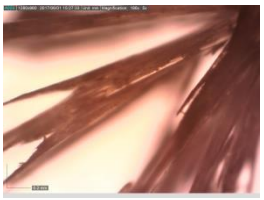
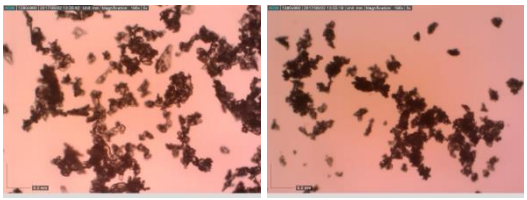
0.50		NA	NA	NA
1.00		NA	NA	NA
1.50		NA	NA	NA
2.25		NA	NA	NA
2.50	NA	NA		

2.75	NA	NA	
3.00		NA	

Note: NA means no data available for analysis.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

The formation of CBZ-SAC co-crystal was investigated using solvent evaporation, cooling crystallization, slurry and stirring method in different solvent systems which are ethyl acetate and formic acid. The XPRD analysis had confirmed that only CBZ-SAC co-crystal in ethyl acetate solvent were successfully formed while in formic acid solvent the crystal formed were only SAC precipitate. From this analysis it shows that CBZ-SAC Form I and Form II had been produced from different ratios and different methods and it have their own melting point based from the DSC analysis. This show that further study in screening is needed for co-crystal formation assessment since there were already many factors proven in affecting the polymorphic formation of the co-crystal such as different methods, solvent and mole ratio. Varies solvent type should be implemented to investigate if the solvent yield CBZ-SAC co-crystal formation and its polymorph.

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