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Approaches to Drying Hydrated Active Pharmaceutical Ingredients (APIs).

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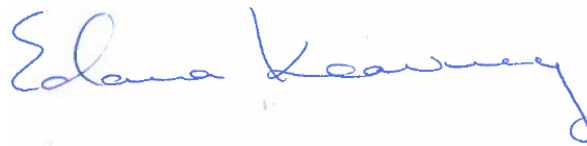
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Edana Keavney

Abstract

The ease with which a patient can take their medication often dictates their willingness to take it. Oral administration is the most convenient route of administration and, as a result, many drugs and medicines are prepared as oral solid dosage forms, with tablets being the most popular. Coupled with this, many pharmaceutical substances are known to exist as hydrates, and an examination of the European Pharmacopoeia revealed that 29% of the compounds are known to form hydrates. ^[1]

For a solid dosage form, drying is an essential unit operation. Process optimisation of drying is an area which has long been under-investigated. Without careful consideration of the drying mechanism of a compound or the level of drying required, over-drying can occur. For the drying of crystalline hydrate compounds, drying is used to obtain a specific moisture profile with the levels of bound and free moisture pre-determined. Commonly, excess, loosely bound moisture, is fully removed, leaving the chemically bound water unaffected. Over-drying and improper drying can cause changes in the hydrate/anhydrate crystalline structure which can be detrimental to the stability, dissolution, bioavailability, and the overall performance characteristics of the drug substance or the dosage form. ^[2]

The work of this project is directed at informing the development of a drying protocol based on pre-determined drying times for hydrated APIs, so that only the loosely bound, unwanted moisture is removed and that the water of hydration remains intact.

Firstly, work was carried out on developing robust methods for the generation of the hydrate forms for both model compounds, carbamazepine (CBZ), and theophylline (THEO), using solid-state analysis to confirm the presence of the hydrate form. To investigate

the effects micromeritic properties, like particle size distribution (PSD), on drying and dehydration, methods were explored to generate batches of hydrated crystals with varying PSD. Standard methods were considered to produce 'medium' sized crystals. To begin, solubility data was collected for both hydrates, with this data being used to inform crystallisation methods. For CBZ dihydrate, the transition to the hydrate form, upon addition to water was fast, with morphological changes occurring instantly. Due to this, milling was used in the downsizing of the crystals. To achieve an increase in PSD, a thermocycling method was used between the isolation temperature of the dihydrate and a maximum temperature that did not negatively impact the hydrate form. For THEO monohydrate crystals, cooling crystallisation processes were used for both increasing and decreasing the PSD. This was achieved by altering the cooling rate, fast cooling rate for small crystals and slow cooling rate for large crystals.

From the previous work, hydrate stability was investigated under various conditions. This was carried out to determine the time taken for dehydration and the conditions which induced it. This work also helped to understand the relationships between the hydrate and anhydrous forms. Stability for both hydrate forms was found at only one set of conditions, 75% RH and 25°C, for which the stability remained for a 12-week period. Examining the effects of PSD, smaller and larger crystals of CBZ dihydrate showed the beginnings of a transition back to the starting material after the 12-week period, via XRPD traces. Also, at the centre point of conditions, at 40% RH and 50°C, larger dihydrate crystals retained 1% of moisture after the first sampling point, which was not seen in the other samples. While this is a difference seen due to PSD, the larger crystal did not preserve the hydrate form. The differences in PSD for THEO monohydrate did not affect dehydration or stability.

This work informed a design space within which drying experiments could be proposed from a place of knowledge as some form boundaries were known.

The DVS Intrinsic was then used for a small-scale drying study. This work showed the possible potential of the DVS for use in small-scale drying screening work. It provided a highly controlled drying environment, one that was very difficult to replicate elsewhere, that could generate real time drying data without the need for sampling the cake which disrupts the cake bed and the drying environment. The drying study involved examining the effects of temperature, % RH, PSD, hold times and increased sample size, on the drying, hydrate stability and the ultimate dehydration of both hydrate compounds. Data from this work included drying kinetics data, drying curves and drying rate data. For both compounds, increased % RH leads to an increase in drying time and decrease in drying rate. By increasing the temperature, this resulted in shorter drying times. Hydrate stability was found when held at $\geq 40\%$ RH. For these samples, the hydrate form only began to dehydrate once the method started a 0% RH step. Below 40% RH, hydrate stability was minimal (<10 min), meaning that dehydration began during the 10% RH or 20% RH step, prior to the start of the 0% step.

The use of a non-ambient XRPD, mimicking the experiments in the DVS, gave a greater insight into the transitions during drying and dehydration. CBZ dihydrate appeared to transition to an amorphous when water molecules associated with the carbamazepine molecule were removed, resulting in a disruption to the hydrogen bonding network between the water and carbamazepine molecules. THEO monohydrate dried to a metastable anhydrous form, which when held for a period, saw a transition to its Form II starting material.

Finally, using the data gathered from the DVS, a convective oven that could control temperature, pressure, and levels of % RH was setup along with the development of predictive drying model for convective drying. DVS data was used to obtain values for critical moisture content (X_C) and convective heat transfer coefficient (h_C) which was the first step in the model. An important aspect to translating the findings of the DVS to oven drying, was understanding how gas temperature, oven pressure and % RH (set on the outside) related to each other, giving a different % RH being experienced on the inside of the oven. The use of the Antoine equation made this understanding possible. With this value and a wet bulb temperature (T_w) calculation for use with a single solvent system, drying times for each hydrate form could be predicted.

From the results of this thesis, predicting the drying times of model hydrate compounds is possible using convective drying and the model detailed in this work. Based on validation experiments for the constant rate period, it was found that the difference between the prediction and experimental data for % moisture content at a specific timepoint was <5%.

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- Keavney. E, Wood, B., Healy. A.M, Glennon, B. *Approaches to drying hydrated Active Pharmaceutical Ingredients*. AAPS 2021 PharmSci 360, Philadelphia, USA (attended online), **October 2021**.

Abbreviations

API Active Pharmaceutical Ingredient

CQA Critical Quality Attributes

CBZ Carbamazepine

THEO Theophylline

DSC Differential scanning calorimetry

XRPD X-Ray Powder Diffraction

PSD Particle Size Distribution

MTBE Methyl tertiary-butyl ether

GC Gas Chromatography

IPC In-Process Control

rpm Rotations per minute

PAT Process Analytical Technology

NIR Near-infrared

MS Mass Spectrometry

IPA Isopropyl alcohol

VLE Vapour Liquid Equilibrium

DVS Dynamic Vapour Sorption

TGA Thermal Gravimetric Analysis

EM EasyMax™ System

DIW Deionised water

PTFE Polytetrafluoroethylene

PVM Particle Vision and Measurement

FBRM Focused Beam Reflectance Measurement

SEM Scanning Electron Microscopy

KF Karl Fischer

RSD Relative Standard Deviation

LOD Loss on drying

M.C. Moisture Content

P&ID Piping and instrumentation diagram

LPM Litres per minute

MSZW Metastable Zone Width

Nomenclature

A	Surface area for evaporation (m^2)
A_{bed}	Cross sectional Area of the cake bed (m^2)
$A_{chamber}$	Cross sectional Area of the drying chamber (m^2)
C_{p,dgN_2}	Specific heat capacity of N_2 ($Jkg^{-1}K^{-1}$)
C_s	Humid heat of air-water vapour ($kg\ water\ vapour/kg\ dry\ air$)
e^{DAB}	Mass diffusivity (m^2/s)
e^k	Thermal conductivity ($W/m.K$)
G	Gas flowrate (L/min)
h_c	Convective heat transfer coefficient (W/m^2K)
h_G	Heat transfer coefficient (W/m^2K)
k_y	Mass transfer coefficient (m/s)
L_e	Lewis number ($-$)
M_0	Initial Mass (kg)
m_s	Mass of dry solid (kg)
M_w	Molecular weight (g/mol)
Nc	constant drying rate ($kg/m^2.s$)
P	Pressure (Pa)
P_c	Critical pressure (Pa)
P_{out}	Outside vapour pressure (Pa)
P_{sat}	Saturated vapour pressure (Pa)
Pr	Prandtl number ($-$)
Q_C	Convective heat flux (W)
Q_R	Radiative heat flux (W)
Q_K	Conductive heat flux (W)
RH	Relative humidity (%)
S_c	Schmidt number ($-$)
T	Temperature (K)
T_c	Critical temperature (K)
T_g	Gas temperature ($^{\circ}C$)

T_s	Solid temperature	(°C)	
T_w	Wet bulb temperature	(°C)	
X	Moisture		content
	(% kg water/kg dry solid)		
X_c	Critical	Moisture	Content
	(% kg water/kg dry solid)		
X_e	Equilibrium	Moisture	Content
	(% kg water/kg dry solid)		
X_0	Initial		moisture
	(% kg water/kg dry solid)		
X^*	Equilibrium		moisture
	(% kg water/kg dry solid)		
Y'	Absolute humidity	(% kg water/kg dry air)	
Y'_{sat}	Saturated humidity	(% kg water/kg dry air)	
Y'_w	Absolute humidity at wet-bulb temperature		
	(% kg water/kg dry air)		
μ	Fluid viscosity	(m^2/s)	
λ	Latent heat of vaporisation	(J/kg)	
θ_c	Constant drying period	(s)	
θ_f	Falling drying period	(s)	
θ_{exp}	Experimental time	(s)	
θ_{mod}	Model time	(s)	

CHAPTER 1. Introduction

1.1. Background

Oral administration is the most convenient route of administration for patients and, as a result, many drugs and medicines are prepared as solid oral dosage forms, with tablets being the most popular choice. The process of drug development is a long and arduous one, with considerable time and money being invested in the development stage. As part of the drug development, the active pharmaceutical ingredient (API) manufacturing process and isolation steps are fine-tuned to produce a cost-effective API that is both efficacious and safe. To ensure the integrity and safety standards of any pharmaceutical product, there are strict guidelines which are set out by regulatory bodies regarding the product's critical quality attributes (CQAs). CQAs include product purity, stability, and impurity profiles, and to achieve required CQAs, process control is paramount. A drying protocol constitutes one important type of process control, as CQAs can be greatly affected by drying. ^[3] For example, the relationship between the absorption/bioavailability of an API and its polymorphic or hydrate/solvate form is an important aspect of the development process, and by not having a product-specific drying protocol, the developmental work, and the product itself may be put in jeopardy.

Regardless of the manufacturing process, as a unit operation, drying is extensively used throughout the pharmaceutical industry. However, it is highly energy intensive and is often costly and time-consuming. Drying is required for the vaporisation of unwanted moisture/solvent and, depending on the drug product, the expense of drying can increase, or decrease based on the amount of solvent to be removed. Especially in cases where there are large

amounts of water, the drying process may incur high costs due to water's high heat of vaporisation.

Throughout the process of bringing a drug from the lab to the pharmacy, there are many opportunities for the solid to undergo changes, often in the form of polymorphic transitions. Polymorphs are crystalline solids which have the ability to exist in different crystal forms or structures ^[4] and the process of changing from one form to another is called a polymorphic transition. These transitions can be the result of improper storage, incorrect shelf life and extreme conditions experienced during manufacturing and/or processing. Different polymorphic forms of the same API can have different physicochemical characteristics such as melting point, solubility, dissolution, compactability etc., and ultimately a change in polymorphic form can have implications with respect to drug product performance. Understanding these transitions can help prevent negative outcomes which can be caused by the physicochemical properties of an undesirable form. There are many examples of this and the resulting recalls of many marketed drugs; in 1988, Tegretol (carbamazepine) tablets were recalled because of both failed clinical testing and differences in dissolution behaviours which was found to be due to a transition from the anhydrate to dihydrate. ^[5] Ten years later, saw the well-publicised recall of Norvir® (ritonavir), an antiretroviral medicine used in conjunction with other drugs to treat HIV/AIDS. Its discontinuation was because of a new, ritonavir crystal structure that was found to be significantly less soluble than the desired form. ^[6] This was caused by conformation polymorphism, a phenomenon that occurs when different conformers of the same molecule appear in various crystal formations. A conformer being an isomer of a molecule which differs from another isomer by rotating one of the molecule's bonds. Again the need for further polymorphic understanding was

demonstrated in the case of Vermox® (mebendazole), where solid-state data showed that imported raw material contained mixes of undesirable solid forms. An inactive or less effective polymorph (Form A) or a mixture of undesired forms (Forms A and B). [7] In more recent years, 60 million tablets of the blood pressure drug Avalide® (a combination of hydrochlorothiazide and irbesartan), were recalled from the market. It was suspected that a less soluble polymorph of irbesartan was present in varying levels, resulting in a slower rate of dissolution and negatively impacting its bioavailability. [8]

Thyroxin, also known as levothyroxine on the market, is a medication commonly prescribed for the treatment of hypothyroidism, a condition characterized by an underactive thyroid gland. There have been many recalls to various manufacturers of levothyroxine, most recently TIROSINT-SOL, which was voluntarily recalled by IBSA Pharma due to the sub potency of the product which was found during stability testing. [9] Stability or degradation issues arise when the drug has degraded over time due to factors like exposure to light, heat, humidity, or improper storage conditions. This degradation can lead to a decrease in potency.

Tetrahydrothyroxin, also known as TETRAC (Tetracarboxylic acid), is a thyroxine derivative that has been studied as an investigational compound. It is a modified form of thyroxine. Issues incorporating hydrated forms of thyroxin and excipients have been investigated where it's been found to result in a transition from a pentahydrate to a monohydrate depending on the excipient used. [10] With issues of sub potency of the marketed drug already being found, the ease at which the drug gains and loses water molecules will be of great interest as TETRAC moves through clinical trials.

This chapter will provide an introduction by first discussing the context and scope of this work, followed by the central research questions of the thesis, aims and overall project structure.

1.1.1. Solid-state properties of pharmaceutical materials

Oral administration is convenient and empowers the patient to be in control of their health care. From this, the manufacturing of solid dosage forms within the pharmaceutical industry requires thorough understanding of the solid-state and physicochemical properties, along with the affects different solid-state forms can have on the pharmaceutical material. Basic characteristics of any solid-state form include a fixed shape or morphology which has minimal translational movement, when compared to a fluid, and strong intermolecular forces. Within manufacturing, pharmaceutical properties such as crystal habit, porosity, hardness, compressibility, and elasticity become important as each can affect the handling, storage, and stability of the product.

Materials in the solid-state can be either crystalline or amorphous. Amorphous forms lack a structured lattice of atoms and molecules, and have only a short range order of molecules due to the intermolecular forces present. ^[11] Crystalline forms have atoms and molecules arranged in a repetitive structure forming a crystal lattice where there is a long-range order of molecules. With respect to crystalline solids, they can also exist in more than one crystalline form, and this is known as polymorphism. This term refers to the ability of a crystalline substance to take on various lattice configurations without changing its chemical composition.

Hydrates and solvates are other known solid-state forms that involve the incorporation of solvent molecules, either stoichiometrically or non-stoichiometrically into the crystal lattice.

When water is inside the lattice, this is known as a hydrate and when other solvents are present, the form is called a solvate. A hydrate may be considered a molecular adduct, meaning it is produced from the direct addition of two or more different molecules, giving rise to a single reaction product that contains all atoms of all components. This makes hydrates distinguishable from polymorphs. For a single component system, specifically polymorphs, temperature and pressure determine the free energy, whereas for hydrates, there is a two-component system involved, and water activity has an impact on the free energy as well.

Depending on the solid-state form of an API, differences in physicochemical properties like melting point, solubility, density, and dissolution rate may be observed. These differences, along with the previously mentioned pharmaceutical properties (crystal habit, hardness, porosity, and compressibility), can be detrimental to the bioavailability, stability and overall performance of a drug substance or dosage form. The most famous reported case of this was for ritonavir, a peptidomimetic drug used in the treatment of the HIV-1 infection.

Thermodynamic stability varies between polymorphs and knowledge of the differing stabilities along with an understanding of the relationship between the different solid phases of a drug substance are the building blocks for a robust crystallisation process. The polymorphic form with the lowest Gibbs free energy at a given temperature and pressure is the thermodynamically stable form at those conditions and can be characterised by having the lowest solubility. For crystalline polymorphs, there are different systems which can be at play: monotropic and enantiotropic systems. In a monotropic system, one polymorph is stable at all temperatures and pressures below the melting point. All other polymorphs of the material are unstable. **Figure 1.1 (A)** shows the

Gibbs free energy diagram of a monotropic system. The graph shows no intersection of the polymorph curves below the melting point, meaning no reversible transition between polymorphs below the melting point occurs. The other system is enantiotropic. In this case, one polymorph (Polymorph B in **Figure 1.1 (B)**) is stable up to a specific temperature, known as the transition temperature, above which there is a form change and another polymorph (Polymorph A in **Figure 1.1 (A)**) becomes the more stable form. From **Figure 1.1 (B)**, the Gibbs free energy curves intersect below the melting point, and this is where the form change would occur. Due to the fact that kinetic factors as well as thermodynamic factors influence polymorphic transitions, even the thermodynamically metastable form might occur under certain conditions. ^[12]

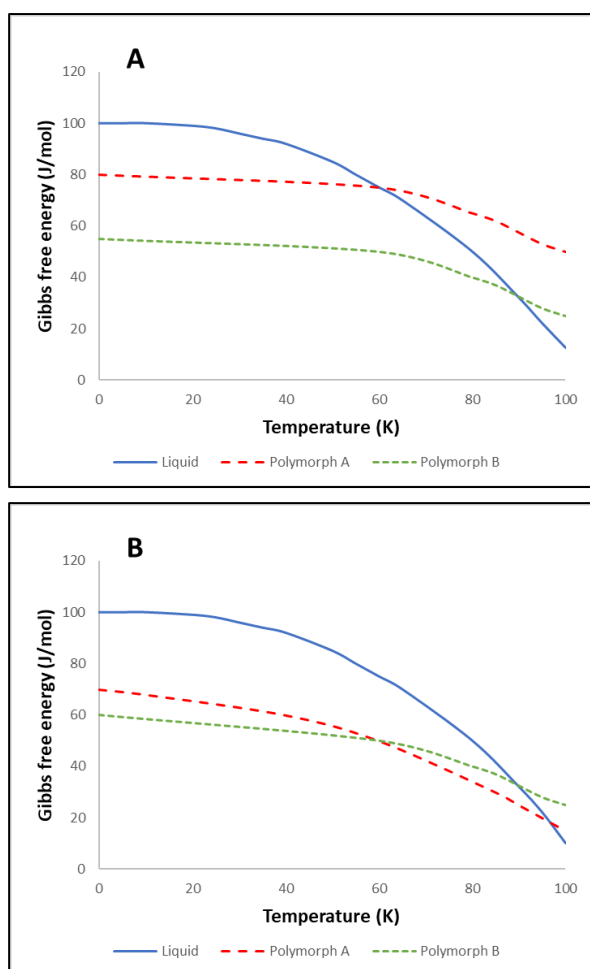


Figure 1.1: Gibbs free energy diagrams for **(A)** monotropic and **(B)** enantiotropic conditions. Adapted from Brittain (2016). ^[11]

1.1.2. Pharmaceutical hydrates

Many pharmaceutical substances exist as hydrates and an examination of the European Pharmacopoeia revealed that 29% of the compounds are known to form hydrates. ^[1] Changes in the hydrate/anhydrate crystalline structure can be detrimental to the bioavailability, stability and the overall form performance of a drug substance. ^[2]

The presence of water within crystal structure can affect internal bonding and its interactions. This in turn changes the internal energies and enthalpy values and, when coupled with changes in entropy, this is what gives rise to the differences in free energy, as

previously mentioned in **Section 1.1.1**. Solubility and chemical stability can also be altered by the presence of water molecules when compared to the anhydrate alone. Hydrate crystals tend to have lower solubility than anhydrous crystals due to the presence of water molecules within their crystal lattice structure. The water molecules are typically bound to the drug molecules in the crystal lattice through hydrogen bonding. When a hydrate crystal comes into contact with a solvent, such as water, the water molecules in the solvent can compete with the water molecules within the crystal lattice. This competition can disrupt the crystal structure and facilitate the release of drug molecules into the solvent. However, the presence of water molecules within the crystal lattice introduces additional intermolecular forces, particularly hydrogen bonding, which can strengthen the crystal structure. These forces hold the drug molecules more tightly within the lattice, making it more difficult for the solvent molecules to replace the bound water molecules and dissolve the crystal. There are many hydrate states – hepta, tri, mono etc. Polymorphism can occur with hydrates, only when the stoichiometry is the same, resulting in a different molecular arrangement.

When dissolution is the rate-limiting step for drug absorption, anhydrous forms often have better aqueous solubility and dissolution rates than hydrates, which may result in increased bioavailability.^[13] Particularly for hydrates, a drug's solubility and rate of dissolution can vary dramatically, in comparison to its anhydrous form. Perhaps because there are fewer sites on the drug molecule available for interaction with water during dissolution, hydrates have a tendency to dissolve more slowly than the anhydrate.^[14] An example of this is theophylline anhydrate dissolving quicker than that of the monohydrate.^[15] Other times, which are less frequently seen, the hydrate form shows a faster

rate of dissolution than the anhydrous form. For instance, erythromycin dihydrate was discovered to have a much faster rate of dissolution than the monohydrate and anhydrous forms. ^[16]

Solubility and stability differences between hydrate and anhydrous form can influence the desired form within a manufacturing process. Many excipients ^[17], antibiotics ^[18] and supplements ^[19], for example, are produced in their hydrated form as they are stable at ambient conditions, provide better bioavailability and/or have more reported benefits of that form when compared to other forms.

Commonly, in a manufacturing process it is advantageous to produce the anhydrate form when its hydrate form is generated in an aqueous solution and when there is a transition from the anhydrate to hydrate form only when held only in high humidity environments. Choosing a preferred form becomes difficult when the hydrated form is formed under low levels of % relative humidity (% RH). For example, carbamazepine transforms to carbamazepine dihydrate when held at humid conditions, above 52% RH. ^[20]

1.1.2.1. Relevance of hydrates in the pharmaceutical industry

Chemical reactions benefit significantly from the hydration process. This is primarily due to the fact that water is a common solvent and is present in many reactions to one degree or another.

For the manufacturing of APIs, hydrates are particularly interesting for a number of reasons, the first being the unique bonding possibilities with the water molecule. Donor and acceptor interactions of hydrogen bonding are possible and it is also possible for such interactions to occur simultaneously, making water a crucial material when developing crystallisation processes. ^[21]

Another point of interest is the fact that water is non-toxic, making it an ideal solvent to work with, especially when generating crystalline material for use within living beings. The permitted solvents are often restricted to water and a small number of bio-compatible solvents. The use of water as a reaction solvent is also preferred when manufacturing materials on a large scale. Pharmaceutical companies must find ways to develop essential medicines sustainably in an era when green manufacturing is more important than ever. It pays to carefully consider the solvents used, as the amounts and toxicity levels of solvents used for processing can greatly affect the size of a company's environmental impact. Finding solvents for reaction, work-up, and product isolation procedures that result in the best overall results for the product and process is a difficult task, so when water is an option, this is an advantageous route to take.

Another point of interest for choosing the hydrate form relates to the presence of moisture in the air and the possibility of spontaneous hydration due to processing or storage conditions. By making the hydrate form the preferred option, the imposition of strict storage conditions can be avoided, and the risk of form change prior to reaching the patient can be minimised.

The chemical stability of a crystalline form, whether hydrate or anhydrous, is determined by the specific compound and its inherent characteristics, as well as the conditions under which it is stored and handled. However, hydrate crystals typically exhibit poorer bioavailability as a result, which is a clear drawback in terms of their medicinal applications, as they are less likely to dissolve in water. ^[22] However poorer dissolution characteristics may be altered through cocrystallisation ^[23] or through the incorporation of excipients. ^[13, 24] In contrast, hydrates are often more compressible and easier to tablet than the anhydrate form, they

are less impacted by wet granulation processes, and are more resistant to the circumstances associated with tablet storage, such as temperature and relative humidity. The choice between a hydrated and anhydrous form of an API is very often a critical factor for solid dosage forms' stability.

1.1.2.2. Classification of hydrates

Hydrate formation involves the incorporation of water molecules into the crystal lattice of an anhydrous material. Water within a hydrate holds a specific place in the lattice, frequently via forming hydrogen bonds with the anhydrate molecules. The stability of any hydrate is governed, in addition to temperature, humidity and pressure, by the arrangement of the molecules, and the intermolecular bonds and also the water activity. Water activity (A_w) is defined as the ratio of the vapour pressure of water in a material at a given temperature (p) to the vapor pressure of pure water (p_0). The value of A_w for a crystallisation medium (solvent mixture), determines the type of anhydrate or hydrate phase that crystallizes when a drug that forms a hydrate is crystallized from mixtures of organic solvent and water. [25, 26] In their solid-states, hydrated forms will be more stable below the dehydration temperature and above the critical water activity, e.g. high humidity or a saturated solution [27], the critical water activity being the value for which the hydrate form only would be crystallised.

The way in which water interacts in the lattice can differ based on the host molecule. The most common way of classifying hydrates is using a structural classification system. This structural classification system categorises hydrates in two way - first, depending on how much water is incorporated, and second, how the water molecules interact with both the host molecule and other water molecules.

With respect to how much water is incorporated into a hydrate - there are stoichiometric hydrates, where a fixed number of water molecules are found in well-defined structures ^[28] and non-stoichiometric hydrates, which have a variable numbers of water molecules in the crystal lattice, with their naming usually indicating the maximum amount of water molecules that can be in the structure. In comparison to stoichiometric hydrates, the variation in water molecules in non-stoichiometric hydrates causes them to be disordered and leads to weaker bonds with the host molecules. The water diffusion or loss from these structures is therefore much easier because of those weaker bonds. ^[29]

The second system of hydrate categorisation involves splitting them into three main groups:

1. Isolated-site hydrates
2. Channel hydrates
3. Ion coordinated hydrates.

In isolated-site hydrates, the water molecules do not interact with one another, with their direct contact being prevented by the drug molecules themselves. An example of this is found with dapsons, an isolated-site hydrate with non-stoichiometric behaviour which is used in the treatment of leprosy. ^[30]

The next and most common type are channel hydrates, where water molecules form tunnel-like structure(s) through the crystal, along a crystal axis and are joined to one another by hydrogen bonds. In these hydrates, one or two-dimensional channels or planes in the crystal structure are filled with water molecules. CBZ dihydrate is a well-known example of a channel hydrate, with each channel containing four parallel and cross-linked chains of water molecules. ^[31]

Channel hydrates can be either stoichiometric or non-stoichiometric. When water fills the tunnels to a defined level, these are considered stoichiometric channel hydrates. When the lattice expands continually over a wide range of humidity conditions, to make room for more water molecules, this leads to non-stoichiometric channel hydrates. When water is removed from a channel hydrate, its dehydrated form tends to be isomorphic, meaning it has the main structural features of the hydrate form, with no observable phase changes occur during hydration or dehydration. Lattice collapse after water removal is more characteristic of stoichiometric channel hydrates, especially for lattices with large channels. The ability of non-stoichiometric hydrates to expand and contract makes them less susceptible to lattice collapse. ^[21]

The last group of hydrates are the ion-coordinated hydrates, where alkali and transition metals directly interact with water molecules. The hydration of several metal ions and their structures are discussed in the literature ^[32] with examples including tetrahedral tetraaqualithium ion and octahedral hexaaquasodium ion being the most common hydrate forms of their respective metal ions.

Dehydration temperatures vary across all three types of hydrates discussed above, with ion-coordinated hydrates generally needing very high temperatures to break the bond between the metal ion and the water molecules. ^[33] Of the other two types, channel hydrates usually dehydrate at lower temperatures when compared to isolated-site hydrates. The higher temperatures used to dehydrate isolated-site hydrates can cause dehydration to be harmful because it forces the molecules in the unit cell to reorganize so that the water can be released.

1.1.2.3. Hydration/dehydration of hydrates and resulting phase changes

Spontaneous water absorption can cause hydrate formation which leads to solid-state transformations between anhydrate forms and their corresponding hydrate form, or even between hydration states. This is a common issue that can lead to undesired differences in the CQAs of the final product.

Undesired hydrate formation can occur when the API meets water during the manufacturing process e.g., during crystallisation, or under high humidity storage conditions. Anhydrous CBZ undergoes such a conversion into the hydrate form (dihydrate), whose altered crystallinity causes a substantial decrease in the drug's bioavailability.^[34] The anhydrous Form III of CBZ is the most potent form and is also the most hygroscopic. Therefore, it is crucial to examine the stability and thermal behaviour of the spontaneously hydrated form, as well as the regeneration of the active anhydrous Form III and the polymorphic transformations involved in this process.

The structure of the material is clearly closely related to the mechanism of hydration and dehydration. A good example of the challenges associated with compounds that are prone to hydrate formation is fluconazole. The hydrated form could be considered preferable for reasons related to both structures, it being similar to the anhydrate was consistent with the ease of hydrate formation, and its kinetics. However, ultimately the hydrated product lowers the drug's solubility and in turn, its bioavailability.^[35] High humidity storage conditions are also known to cause hydrate formation, for example in the case of commercial theophylline tablets.^[36] In the presence of hygroscopic formulation ingredients and during

storage at 90% RH, THEO monohydrate needle-like crystals formed on the tablet surface, which was confirmed by XRPD analysis.

Given that some APIs experience water desorption-induced phase changes, this kind of adverse process should be taken seriously and carefully considered when determining process and storage conditions. Dehydration of hydrated APIs can occur due to changes to ambient temperatures and/or humidity levels, mechanical forces being applied, or during storage. This can then result in a phase change to an anhydrous form (crystalline or amorphous) with different physicochemical properties e.g., solubility, hygroscopicity and properties affecting tableting. Understanding the mechanisms of dehydration for hydrated APIs and conducting investigations into their transitions are both crucial for drug development. The mechanisms behind the dehydration process are strongly associated with the intermolecular interactions and ultimately, the substance's structure. ^[37] The type of hydrate (isolated-site, channel etc.) is known to not only influence hydrate stability but also its dehydration temperature. ^[21] It is common to use the dehydration temperature, the temperature at which water begins to leave the surface of the crystal or from within its lattice, to determine whether water is loosely or tightly bound. Low onset temperatures for endotherms seen via Differential Scanning Calorimetry (DSC) analysis relates to loosely bound moisture, as reduced heat/energy is required to break bounds, whereas higher temperatures are required to break the tighter bounds. However simple evaluation of dehydration temperature in this manner ignores other factors such as intermolecular forces and the ratio of water to drug molecules within the hydrate crystal lattice. ^[37]

Channel hydrates are the most common type of hydrate. Comparatively to isolated site hydrates, ^[13] dehydration of channel hydrates often takes place at lower temperatures and can result in

sample amorphization. ^[38] In some situations, crystallinity can be maintained after water loss even though the collapse of water channels results in severe structural alterations, an effect that can be reversed by exposing crystals to high humidity. ^[39] The crystal structure can remain intact with minimal structural changes when water molecules do not play a significant role in the structure's integrity. ^[40-42]

The mildronate dihydrate structure provides a prime example for understanding a structural dehydration mechanism of a channel hydrate. ^[43] Its dehydration process is one-step and results from the presence of very big channels, which cause a loss of all the water at once rather than in stages, clearly demonstrating the connection between structural factors and the kinetics of dehydration.

For other channel hydrates it has been reported that dehydration can cause cocrystals to form between the anhydrous API and a second formulation component, mediated by the released water. ^[44] The reported studies involved two systems: THEO monohydrate and citric acid compressed into tablets under conditions that preserved the hydrate form, and CBZ dihydrate and nicotinamide compressed into tablets in a similar manner. Dehydration, by storing at 40 °C, saw the first system transition from the monohydrate to the anhydrous form of THEO, followed by the formation of a THEO-citric acid cocrystal within intact tablets. Humidity readings of the tablet headspace were high and remained constant, suggesting that the water released during dehydration was a mediator of cocrystal formation. Under the same conditions, CBZ dihydrate was found to dehydrate quicker (within 1 day) and formed a cocrystal with the nicotinamide, once again suggesting a water-mediated cocrystal formation process. From additional work on the CBZ system, it was also suggested that the water-mediated co-crystallisation was likely due to the materials' tight packing in

the tablet, allowing for close contact between the reactants, which promotes the formation of cocrystals. Co-crystallisation was not observed when components were simply physically mixed, and not compressed into tablets. Additionally, it is known that the compression process results in lattice disorder, which could enhance reactivity. ^[44]

Both hydration and dehydration mechanisms can be influenced by the number of steps in the process and whether an intermediate is formed. An example of multiple step dehydration is seen with the nonsteroidal anti-inflammatory drug, diclofenac (used in its sodium salt form) which is an analgesic. It was discovered that the 4.75-hydrate form dehydrates in two stages. Only water molecules that are not coupled to Na⁺ ions are lost during the first stage, resulting in the creation of the 3.5-hydrate while preserving the alternating layered structure of the hydrate crystal. ^[45] The phase transition to the anhydrous form occurs during the final dehydration step, where 3.5 molecules of water are lost. Along with this loss of water, there is also significant structural reconstruction. This multi-step dehydration mechanism was confirmed through the use of solid-state analyses such as DSC and X-ray Powder Diffraction (XRPD). ^[45]

1.1.2.3.1. Processing-induced dehydration

Dehydration can also be influenced and impacted by the preparation and processing of the material e.g., milling and/or grinding and other mechanical forces being applied. Particle size reduction is commonly used to improve the dissolution rate of poorly soluble crystalline drugs. It was discovered that while the dihydrate form of CBZ was the most compressible, it was also the least stable once compressed, with 50% of the hydrate form transforming to the Form III anhydrate. ^[46] Using DSC, it was also discovered that structural changes in the crystal lattice, due to

grinding, resulted in a lower dehydration temperature. An increase in grinding time led to both a decrease in dehydration temperature and water content, suggesting that grinding caused water molecules to be less bonded and more easily released from the crystal lattice.

Another example of processing-induced dehydration relates to the hydrated form of 7-methoxy-1-methyl-5-(4-(trifluoromethyl)phenyl)-[1,2,4]triazolo[4,3-a]quinolin-4-amine, which was investigated as a potential treatment for dyslipidemia. At ambient temperatures, it was discovered that the dehydration rate for micronized portions of the hydrate was more than two orders of magnitude higher than non-micronized material. ^[47] The findings showed that accessibility to the outside environment, which is mostly influenced by particle size and morphology, controls the difference in dehydration processes between non-micronized and micronized samples. It was discovered that narrow and staggered channels were related to the variations in dehydration kinetics. The cleaving of slip planes, which are roughly perpendicular to the long axis of the water channels, is thought to have been induced by micronization. This causes water to have an easier exit route, drastically varying the water sorption/desorption characteristics between the samples. ^[47] A full suite of solid-state analyses was used to conclude these findings, once again showing the importance of full characterisation of all forms, especially after process preparation.

To gain insight into the dehydration mechanisms encountered during processing, it is critical to understand the dehydration behaviour of an API. Producing a final product with well-defined physicochemical and pharmacological qualities is the primary objective of the pharmaceutical industry.

1.1.2.4. Drying of hydrates

Controlling an APIs solid-state while it is being dried is one of the pharmaceutical industry's most difficult undertakings. If the drying process is not effectively controlled, the preferred solid-state form and particle size may not be suitable after drying. When hydrates are involved, either over drying or under drying can be critical, depending on whether the hydrate form or the anhydrate form is chosen for manufacture. Control over the ultimate solid-state form is more challenging when an API exists in numerous anhydrous forms.

The first example used to illustrate the issue of drying hydrated APIs is that of a hemi-hydrate reported as “Compound D”.^[48] The initial concern relating to drying of the hydrate was amorphous formation upon drying. To overcome this, drying under humid nitrogen conditions was found to preserve the hemi-hydrate state and avoided dehydration. While recovery of the hydrate form was also possible by rehydrating in humid conditions, if over-drying occurred, an ideal process would aim to avoid this additional step. Over-drying can form a destabilized crystal structure and that could increase its sensitivity to mechanical forces. If the rehydration step did not happen quickly enough, it could eventually lead to the formation of amorphous content that, if formed, would make the transition back to the hemi-hydrate impossible.^[48] Off-line analysis revealed that neither the hemihydrate nor the anhydrous form tended to generate amorphous material, but undesirable effects may be anticipated and prevented by planning ahead and understanding dehydration mechanisms.

Another example is of SAR474832, which is reported to be a combination of a typical non-stoichiometric channel-hydrate and a stoichiometric channel hydrate with strongly bound water.^[49]

Here, investigations were carried out to find drying techniques that minimized and controlled the production of amorphous content while also avoiding unintended phase changes brought on by over-drying. ^[49] In order to understand potential phase transitions and their boundaries at low humidities and high temperatures, solid-state analysis was first carried out on the hydrate forms. Raman results were coupled with Modulated DSC results (pin holed samples) as they could be compared to the drying oven where water is continuously purged and doesn't remain trapped with the sample (as is the case with a hermetically sealed MDSC samples). Based on these analyses, it was predicted that no amorphous content would form when the hydrate was dried at temperatures between 30 and 80 °C, but that an undesirable transition from Form I hydrate to Form II hydrate would occur at temperatures over 60 °C. Initial drying experiments within the oven found that when the Form I hydrate was dried between 20-30 °C, if the sample cake had a water content of >10% w/w, the % RH level inside the oven stayed above 30% RH until the desired water content of the hydrate, 6.5% w/w was reached. ^[49] At this point, the relative humidity level decreased below 10% RH which facilitated the undesired transformation to Form II hydrate. When the material was again exposed to ambient humidity, it quickly absorbed the water that was lost, returning to Form I hydrate after passing through Form III hydrate which acts as an intermediate. This back-conversion, from the undesirable Form II to Form I hydrate by rehydration, is completely reversible, although the length of time it takes to complete depends on the humidity of the surrounding air and how long the material is exposed to it. This level of unpredictability is not ideal in a production process; therefore, it was deemed necessary to avoid over-drying. To protect the desired hydrate throughout drying, it was ultimately necessary to maintain a high %

RH environment. This was accomplished by adding water reservoirs to the oven, which was proven to maintain the % RH above 30% RH.

1.1.3. Drying as a unit operation

Drying involves two processes working together, heat and mass transfer. Heat is transferred to the wet cake by one of the following: convection, conduction, and radiation. Regardless of the type of drying, the mechanism of heat transfer results in a temperature gradient evolving within the solid, while evaporation occurs from the surface. Mass is then transferred as moisture/solvent migrates to the surface of the cake and then leaves the cake as a vapour, moving into the carrier gas of the chosen dryer. ^[50] For vacuum drying, mass transfer is facilitated by the pressure difference caused by creating the vacuum. Factors like temperature, pressure, and gas flow over/through the solid affect both heat and mass transfer. This determines a solid's drying rate. Typical drying rate profiles are often split into two distinct stages: the constant rate period and the falling rate period.

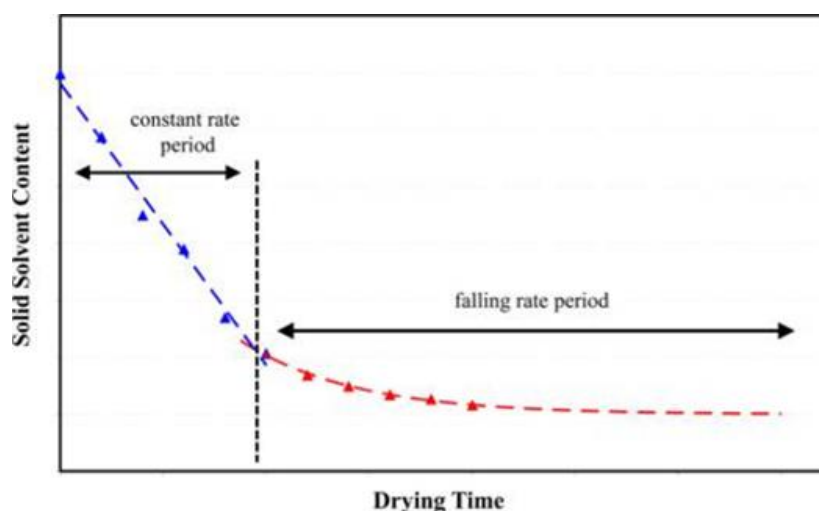


Figure 1.2: Typical Drying Curve. ^[51]

During the constant rate period, moisture is being brought to the surface at the same rate it is being evaporated, maintaining

saturation. In this period, the solid's surface takes on the wet bulb temperature, this being defined as the temperature at which evaporative cooling occurs on the material's surface, bringing the air to saturation at that temperature. As the amount of moisture to be removed decreases during the falling rate period, the solid's temperature approaches the medium's dry bulb temperature, which is essentially the air/gas temperature within the dryer. The wet bulb temperature is determined by a combination of the dry bulb temperature and % RH for an environment, with the wet bulb temperature being lower than the dry bulb temperature, due to the evaporative cooling effect. The amount of evaporative cooling and difference between wet and dry bulb temperature depends on the humidity of the air.

The drying unit operation is a crucial step in the manufacturing of final drug products and is influenced by many parameters. Its importance should not be underestimated. To develop a robust drying protocol for an API, there are three key elements which need to be understood: chemical and physical stability, control over the physical properties and the drying kinetics of the API itself. ^[51] When optimising processing parameters, one must consider how each of these elements may be affected by another. By investigating only one of the elements, the possibility of introducing risk is heightened and this can lead to failures in the product's CQAs. Drying is complex; not only are the three elements important, but it is also necessary to determine how they interconnect with the other parameters encountered in any drying protocol, e.g. temperature, dryer type and level of agitation (if any).

[51]

Process optimisation of drying is an area of study which has long been under investigated. Despite the intensive work which goes into the optimisation of a crystallization process, this can all be

negatively impacted if the drying profile of the sample is poorly understood and/or poorly controlled. Time should be taken to fully understand the solid product and what is to be accomplished during drying, whether that is providing stability for one solid-state form or preventing the formation of another. Through this project, it is intended that a drying platform will be developed which will provide improved control over this critical process, focusing on drying of hydrates specifically.

1.1.3.1. Convection

The most frequent drying method is convection. Heat is provided by hot air or gas flowing over the solid's surface. Heat is delivered to the exposed surface of the material via convection for evaporation of undesirable solvent/moisture, with the evaporated moisture being transported away by the drying medium. ^[50] Air is often used in the drying of wet solids, however inert gases such as nitrogen are preferred because it not only prevents product oxidation but also enables the safe drying of organic liquids. It's also possible to use combustion gases like natural gas, propane and diesel oil in pulse combustion dryers ^[52] and also in types of rotary dryers. ^[53]

Convective dryers are also known as direct dryers since the material to be dried is in direct contact with a hot gas which provides the heat necessary for evaporation. Fluidized beds, flash rotary, spray dryers, and even tray dryers are examples of convective dryers.

1.1.3.2. Conduction

In conduction drying, heat for evaporation is provided by a heated surface that is either stationary or moving. Because heat is given to the material indirectly, through the heated surface, these dryers are also known as indirect dryers. ^[50] The moisture that is evaporated is taken away by a vacuum or a stream of gas that is

primarily a moisture carrier. To minimize the evaporation temperature for heat-sensitive materials, drying under vacuum is used. While vacuum drying is an expensive option, not only does it prevent degradation of heat sensitive materials, but by lowering the evaporation temperatures of materials in general, this reduces the energy consumption of dryers (running at lower temperatures for shorter time periods). The thermal efficiency of conduction dryers is higher than convective dryers.^[50] Examples of an indirect dryers include paddle dryers, rotary dryers with steam tubes or drum dryers.

1.1.3.3. Radiation

There are many sources of electromagnetic radiation with wavelengths ranging from the solar spectrum to microwaves. Solar radiation does not penetrate beyond the skin of a material which absorbs only part of the incident radiation, depending on the wavelength.^[50] Infrared radiation is commonly used for drying water-based inks and coatings and uses a wavelength of 0.78-1000 mm, being a longer range than that of the visible light region, of 0.4-0.7 mm. Microwave dryers are an example of radiation dryers used in the pharmaceutical industry. Industrial dryers that use microwave technology use high frequency electromagnetic waves to penetrate the material and mobilize the molecules from within, enabling fast drying^[54], especially in the case of water removal, as it experiences quick energy absorption and rapid evaporation. Microwave drying is considered to be a clean option, with no gas emissions or residuals. It is highly controllable and tends to be related to lower running costs, based on the short drying times. These dryers have also proven to be an ideal option for removing small amounts of remaining moisture during the falling rate period^[54].

1.1.4. Drying of pharmaceutical materials

Most pharmaceuticals and fine chemicals must be dried before being packaged. For pharmaceuticals, drying is an important process whether it's preparing material for further processing or as a way of reaching a final moisture content level appropriate for tableting or even storage. This moisture content is mostly determined by storage and stability considerations. The drying time and conditions are determined by the final desired moisture content.

The effects of dehydration on hydrated APIs have been discussed in **Section 1.1.2.3**. Where the hydrate is the desired form, unwanted phase transitions and their effects on stability and bioavailability need to be avoided. High temperature, lower pressures or the presence of a solvent vapour can cause dehydration leading to crystal defects that can contribute to the removal of hydrate water. ^[55] To maintain the proper polymorphic form or hydrate/solvate, conditions may need to be carefully controlled. ^[56] When excessively low relative humidity levels are being avoided, the carrier gas can be recycled. By adjusting the condenser temperature, the humidity within the loop can be controlled. ^[57]

1.1.4.1. The use of vacuum in drying

Vacuum drying is typically used to dry hygroscopic, heat-sensitive and/or toxic materials. By creating a vacuum, the chamber pressure can be reduced below the boiling point of water (or other solvent), which will cause the material to dry. For a variety of reasons, from product quality to safety, it may be necessary to avoid using excessive heat when drying powder.

Freeze drying is a type of extreme vacuum drying in which water (ice) sublimates directly into water vapor at a temperature below the triple point of water. Although the amount of heat required for sublimation is significantly less than that required for evaporation.

1.1.5. Dryers

Once the drying conditions needed for a specific compound are known, choosing the correct dryer to achieve this can be considered.

1.1.5.1. Choosing a dryer

Choosing the right dryer for a job is crucial, but it can be challenging. There are a few things to think about. To begin, consider how much material needs to be dried; this will assist in determining whether a batch or continuous dryer is best. Material factors such as Particle Size Distribution (PSD), moisture content, porosity, and temperature sensitivities will all play a role in picking the dryer. ^[50] CQAs of the final product will also play a role in what dryer is chosen. Requirements on particle size, shape and/or moisture content may need to be met prior to batch release. Regulatory bodies enforce these requirements to link the quality attributes to clinical safety and efficacy and having a robust process, including drying, is key.

Another consideration is the material's hazardous nature and the potential for safety difficulties when handling a toxic, explosive, or oxidizing substance. The best approach to assure safety when drying certain hazardous materials is to remove something from the fire triangle. Because the material to be dried is the fuel and heat is necessary for drying, the only alternative is to remove oxygen, which could be accomplished via a closed-circuit setup.

Another aspect to consider is cost, whether it's associated with the manufacturing environment, energy usage, or the availability of the preferred energy source.

The first way to classify dryers is based on the method of heat transfer, namely, (1) convection, (2) conduction, (3) radiation. Dryers are often also categorized by their mode of operation, which divides them into batch and continuous alternatives. In API manufacturing processes, typical throughputs are low, and batch processing is still the preferred option. Continuous dryers are advantageous in situations where production rates are high, and there is an emerging trend of integrating small-scale continuous dryers for primary and secondary manufacturing. The type of drying vessel is another subcategorization that can be made; and tray, rotating drum, fluidized bed, pneumatic, or spray dryers are all available. Dryer classification may also be made based on the physical form of the material to be dried. Tray, rotary, vacuum and freeze dryers are typically used for particulates and granular feeds. For slurries and suspensions, drum and spray dryers are commonly used. While not common in the pharmaceutical industry, the drying of sheet-form material (e.g. textiles) can be done in tunnel dryers or steam-heated cylinder dryers. ^[58]

1.1.5.2. Common types of industrial dryers and their descriptions

1.1.5.2.1. Convection dryers

- Tray dryer – The damp material is equally distributed over shallow trays, with air flowing over each shelf. The reheating of air after it has been cooled by the evaporation of moisture from the solid is aided by either electrical elements or steam heated pipes.

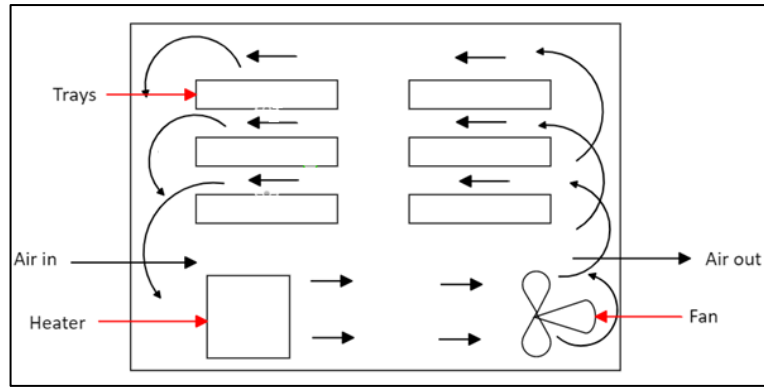


Figure 1.3: Schematic of a batch tray dryer. Image adapted from literature. ^[58]

- **Spray dryer** – By atomizing a liquid into droplets, this dryer provides a high surface area for both heat and mass transfer. The droplets are then blasted into a stream of heated gas, which causes each droplet to solidify. The attached cyclone chamber has adequate air circulation, which promotes heat and mass transfer and allows dried particles to be separated by centrifugal motion. Spray drying has been proven to be effective at a variety of feed rates.

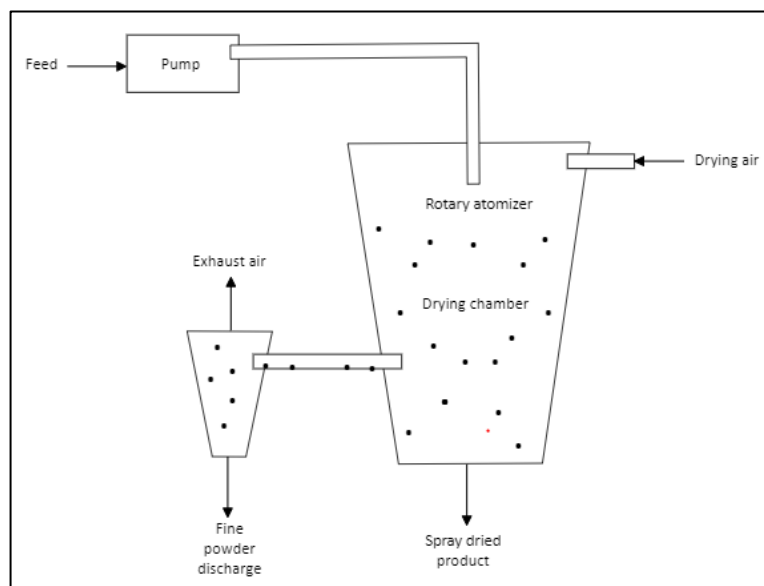


Figure 1.4: Schematic of a conventional spray dryer. Image adapted from literature. ^[59]

- Rotary dryer - Heating of the solid and evaporation of solvent occurs as hot air or gas passes through a cylinder and subsequently transfers to the product. In convective rotary dryers, the product is dispersed in the hot air or gas using lifting flights that are evenly spaced and welded to the cylinder. The varied shapes of the lifting flights ensure that the wet product cascades from the top of the cylinder exposing it to the hot gas flow.

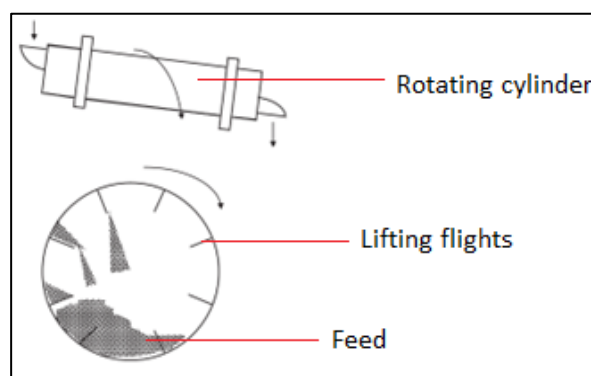


Figure 1.5: Schematic of a general rotary dryer. Image adapted from literature. ^[60]

- Fluidised bed dryer – Fluidization occurs when a mixture of solids and gas behaves like a liquid, with solid particulates being suspended and moved around in the gaseous phase. The hot air and particulates make good contact, allowing for rapid drying. The vessel is supported by a net, through which hot gas travels. The material to be dried is then placed in the vessel.

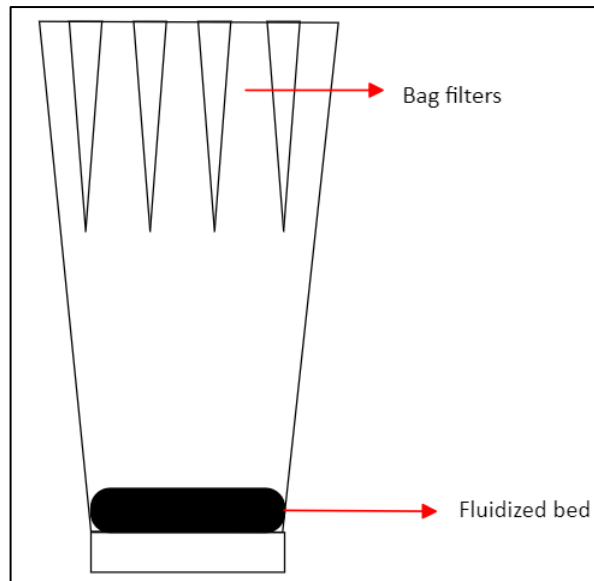


Figure 1.6: Schematic of batch fluidized bed dryer. ^[61]

1.1.5.2.2. Conduction dryers

- Vacuum tray dryer – Trays separate the space within the oven, increasing the surface area for heat conduction. A vacuum pump is attached to the oven, which is airtight to lower the pressure. This system involves the indirect application of heat to a material, under reduced pressure levels. Due to these conditions, the boiling temperature of the solvent is lowered and drying occurs faster than a non-vacuum tray dryer.

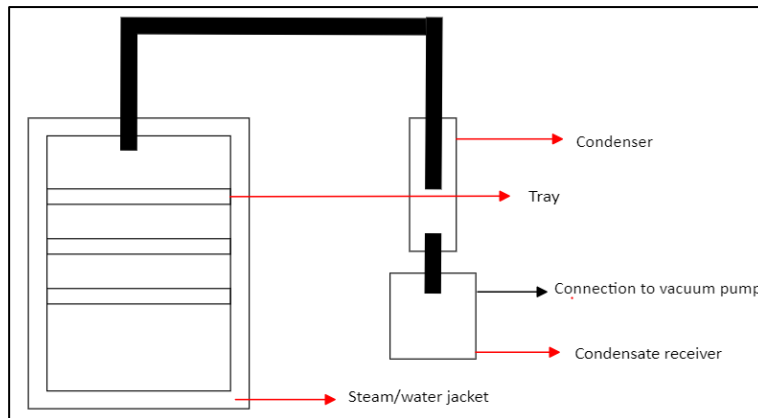


Figure 1.7: Schematic of a vacuum tray dryer. Image adapted from literature. ^[62]

- Drum/roller dryer – These dryers are commonly used for the drying of liquids, suspensions, and slurries. Internally, the drum is heated by steam and rotated. Either a thin layer of the material to be dried is put on the surface of the drum using applicator rolls, or the drum is set inside a trough filled with the non-settling liquid slurry. The latter is known as the ‘dip feeding’ and is the simplest method. ^[60] Drum dryers can either be single or double drummed (two drums rotating in opposite directions) and can be operated at atmospheric pressure or under vacuum.

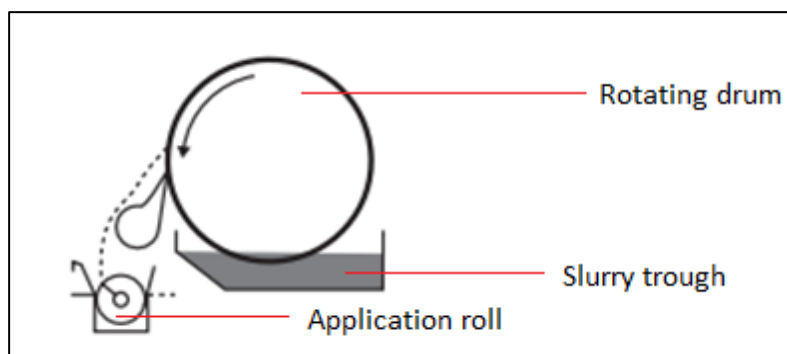


Figure 1.8: Single, dip feed drum dryer. ^[60]

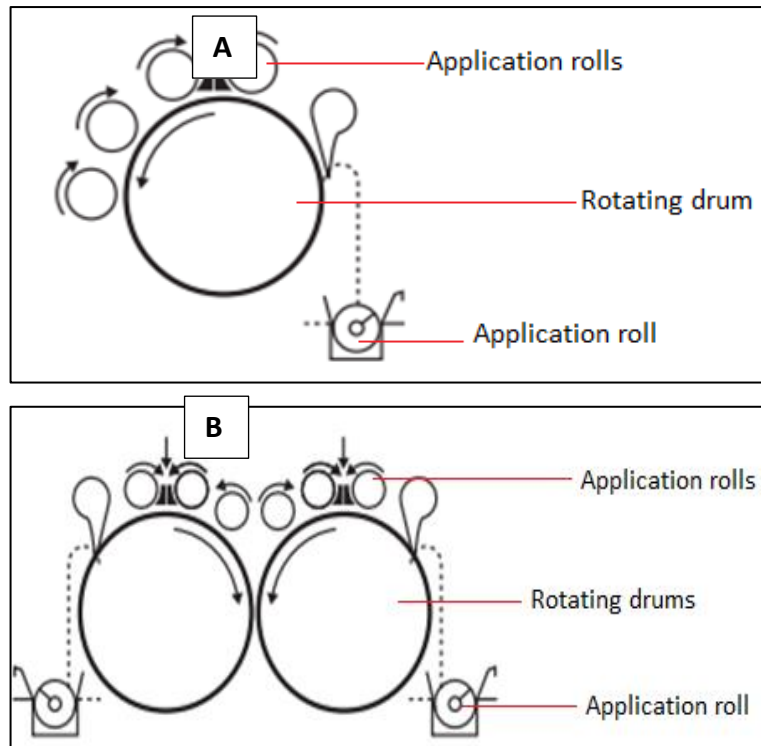


Figure 1.9: (A) Single drum dryer with application rolls, (B) Double drum dryer with application rolls. ^[60]

- Agitated bed dryer - They consist of a vessel or chamber equipped with an agitator that promotes the movement and mixing of the drying material. The agitator helps to break up clumps or agglomerates, ensuring uniform drying and preventing the formation of hotspots. These dryers utilize a combination of heat, airflow, and mechanical agitation to remove moisture from the product. The agitated bed dryer offers several advantages, including efficient heat and mass transfer, short drying times, and the ability to handle a wide range of materials.
- Steam rotary dryer – These are built similarly to a normal rotary dryer, shown in **Figure 1.5**, consisting of a metal cylinder with internal flights. These dryers use steam tubes that line the inside of the rotating cylinder, which increases the thermal efficiency. The material is mixed to aid uniform drying using the tubes and several radial flights, with most

of the drying taking place when the material falls while rotating. The benefits of this dryer include a large processing capacity, quick drying times, and low energy consumption.

- Freeze dryer – Freeze drying is frequently used for very heat sensitive materials. Water is eliminated via sublimation, transitioning from a solid to a vapour, after the original liquid solution or suspension is frozen and the pressure above the cake is decreased. This reduction in pressure is referred to as the primary drying step where 95% of the water is sublimed. The secondary drying phase aims to remove the remaining, unfrozen water. ^[63] Here, the temperature is raised above that of the primary phase and the pressure can also be reduced.

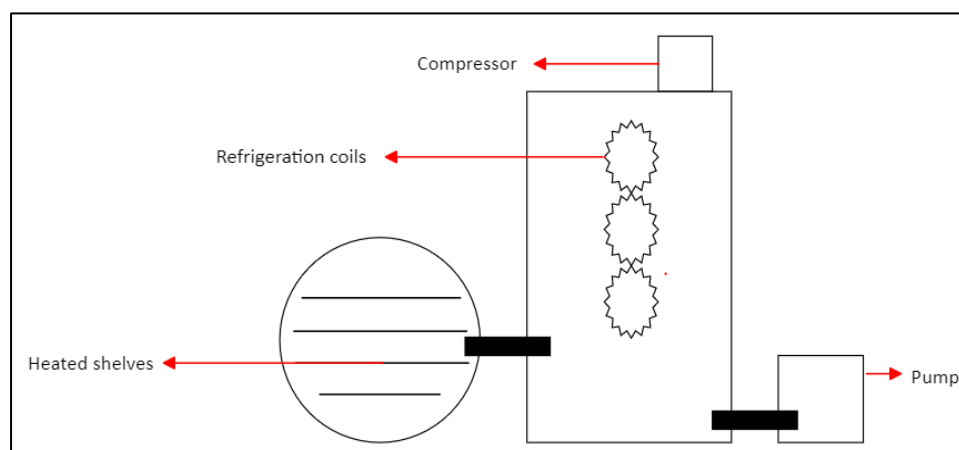


Figure 1.10: Diagram of a general freeze dryer. Image adapted from literature. ^[63]

1.1.6. The impact of drying on powders

This section will explore the range of adverse effects that may be caused by drying, on the stability, structure, and overall performance of pharmaceutical APIs. There are no lab tools which

can be used to predict these effects or predict issues when a process is scaled-up. To develop the best drying protocol for a compound, having a deep understanding of the associated drying kinetics is key to unlocking the control of the entire process.

It has been demonstrated how careful consideration of the three principles of drying: chemical and physical stability, drying kinetics, and physical properties, can help to optimise a drying protocol.

The drying of an ethanolate compound (an ethanol solvate of an API) ^[64] was investigated in order to collect drying kinetics data using a laboratory integrated sorption chamber, with the aim of predicting drying times. The sorption chamber was a modified vacuum oven which allowed for temperature and pressure control along with the incorporation of Raman and mass spectrometer to track drying. It was found that the drying process could be broken into three stages: the evaporation stage, transitional stage, and the diffusion stage. The first stage in the process involved rapid evaporation of the wash solvent, methyl t-butyl ether (MTBE), with very small amounts of residual ethanol also being removed. This stage was controlled by the heat transfer to the cake from the dryer. After 9 min, evaporation finished, and the surface of the API was dry of MTBE. A point of inflection was seen on the drying rate graphs, separating two lines with different slopes. This signalled the beginning of the second stage, the transitional stage. Here, unbound, surface ethanol was removed from the ethanolate by evaporation. The slopes of the two lines differed due to the MTBE and ethanol's different boiling temperatures and heats of vaporization, with the former being lower. This transitional stage acted as a bridge between the fast surface evaporation and the start of the slower process of diffusion drying. During this stage, the rate at which the ethanol was removed from within the crystal lattice was greatly reduced. This is due to higher activation energies

needed to break the non-covalent bonds within the ethanolate structure.

Investigations into the effects of drying the ethanolate over a temperature range of 50–90 °C found that Raman spectra remained unchanged after the removal of ethanol from the crystal lattice. GC was utilised for the assessment of chemical and physical control by quantifying residual solvents. This confirmed that MTBE was fully removed during the evaporative stage as it was undetected through Gas Chromatography (GC). In-process-control (IPC) testing showed that after drying, each sample had near negligible amounts of ethanol present, however, during the diffusional phase of drying, higher temperatures were more efficient at completely removing ethanol. The first two stages may be considered constant rate periods ^[51] and the rate of removal of solvent is controlled by the heat transfer from the equipment wall (jacketed) to the first layer of solids, meaning it is equipment dependent. These stages also represent the loss of unbound moisture from the solid. At increasing temperatures, the durations of the first two stages did not change drastically. However, the diffusion stage had a strong temperature dependence, and there were noticeable variations in drying times. The time spent in the diffusion stage at 50 °C was 1370 min, whereas at 90 °C it was greatly reduced to 49 min. Predicted drying times, via modelling, found good agreement with experimental results at the higher temperatures of 80 and 90 °C, however thermal stability was jeopardised at these temperatures. Based on this, it was concluded that a drying temperature of ≤ 70 °C was to be used; this ensured a manageable drying time (<8 hr) and maintained product stability.

Other work reported in the literature examined how different types of dryers affect the physical properties, such as the tendencies for agglomeration or attrition, of an API with two different median

particle sizes (5 μm and 40 μm).^[65] Increasing the drying temperature within a rotary dryer from 50 °C to 70 °C, when vacuum levels, rotary speed and batch size were kept constant, gave differing results for the two different sized materials. The larger particles experienced a 50% reduction in drying time at 70 °C compared to 50 °C (from 3600 s to 1800 s), which is in line with previous literature.^[50] Increasing drying temperatures, increases the rate of removal of moisture which, in turn, decreases drying times. However, for the smaller particles, there was no significant change in drying times at different drying temperatures. An investigation of the effects of pressure in the drying process showed that reduced pressure gave reduced drying times and an increase in the drying rate. This particularly impacted the larger particles, where it was shown that the drying time could be halved with reduced pressure, even though the initial moisture content of the solid dried at the reduced pressure (2 kPa) was approximately 8% higher than that dried at the higher pressure (50 kPa). Finally, the impact of rotary speed was investigated, and it was found that when the speed was increased from 10 to 240 rotations per minute (rpm), decreases in drying times were seen for both sizes of particles (5 μm and 40 μm). Drying times of the smaller particles was reduced from ~10500 s to ~5000 s. and for the larger particles, drying times went from ~4250 s to ~2400 s. The rotary dryer speed was found to play a key role in the drying efficiency, whereby increasing surface exposure, led to an increase in heat and mass transfer rates and reduced drying times. By increasing the rotary speed, a reduction in particle size for both sets of particles was evident, due to attrition. This must be carefully balanced in instances where attrition occurs and is an undesired effect. For the API in this case study, the overriding parameter which had greatest impact on drying was found to be the vacuum level applied. This

resulted in the most significant changes compared to the drying temperature, initial water content and rotary speed.

The drying of a different crystalline hydrated API was shown to be affected by differences in equipment and drying conditions. ^[66] Processing of the material was unsuccessful at manufacturing scale, as insufficient solvent removal was evident, and this prevented the desired conversion from the hydrated Form I to a weakly crystalline Form II taking place. To investigate these issues, small scale flow cells were equipped with Process Analytical Tools (PAT), including Near-Infrared (NIR) and Mass Spectrometry (MS), to better understand how drying was taking place. The failing and passing results shown at large scale were effectively replicated using this small-scale experimental setup. It was demonstrated that successful drying could be accomplished under very moderate drying conditions (20 °C and at 200 and 350 mmHg), however the use of more aggressive drying conditions and recycled nitrogen contributed to solvent trapping. The use of aggressive drying conditions (29 °C) within the small-scale flow cell caused the water content of the solid to decrease sharply, which in turn caused early form conversion to occur before the isopropyl alcohol (IPA) had sufficient time to be fully removed. The use of moderate drying conditions allowed for the correct form and target IPA levels to be recovered. It was deduced that by manipulating the temperature and pressure of the system, the water content could be better monitored, to slow down its removal and the onset of the form change and, in turn, give more acceptable IPA solvent levels. Findings also showed that, although the use of recycled nitrogen might have prevented the removal of IPA, it was not the main factor that led to solvent trapping and the use of fresh nitrogen gave results which showed that the IPA level was reduced but was still failing specifications. The authors also showed how the use of PAT

was effective at gathering useful data which allowed for a greater understanding of the solvent trapping issues. NIR was useful in tracking water and IPA signals during drying and in obtaining weight % values for both. MS was also used in studies comparing aggressive and moderate drying conditions, which allowed solvent content levels to be measured in the vapour phase. Such data helped to provide an understanding of the drying process, its effects on the final product and allowed for drying optimisation, by altering conditions which minimised the risk of an undesired form conversion. The drying of a different crystalline hydrated API, , was shown to be affected by differences in equipment and drying conditions. ^[66]

Another case study ^[67] detailed a drying process which was shown to have a major impact on the form stability of an “organic monohydrate” obtained via crystallization from 80/20 (w/w) isopropyl alcohol/water. Drying of the material at a factory-scale was required to generate the desired dry monohydrate, while avoiding undesired dehydrated crystalline species and an IPA solvate. The tendency of a hydrate crystal to continue to lose water once residual free solvent has been removed, is an important factor to evaluate when dehydrated forms are undesired. The use of NIR spectroscopy to determine drying endpoints has been widely discussed ^[68-70], however NIR probes tend to be expensive and have shown inaccuracies in differentiating between free and bound water in the cake or the water content levels within the solvent vapour. This particular case study ^[67] detailed an interesting and inexpensive option, using a dew-point hygrometer which tracked the water content of the vapour effluent stream. Preservation of the monohydrate through drying required the prevention of solvate formation and over drying. By determining polymorphic boundaries and generating Vapor-Liquid Equilibrium (VLE) data, an

appropriate wash solvent ensured that the desired hydrate form was preferentially formed over the solvate. This chosen wash solvent allowed for the wet cake to consistently have a % water content higher than 8%. To ensure that over drying, leading to a dehydrated or anhydrous crystalline structure, was prevented, solid-state analysis was utilised. Dynamic Vapour Sorption (DVS) demonstrated the stability of the monohydrate with respect to the relative humidity as a function of temperature and conclusions from this study suggested that a humid nitrogen sweep would ensure a sufficiently 'wet' environment to prevent dehydration. Thermal Gravimetric Analysis (TGA) was used to investigate the drying kinetics under a dry nitrogen sweep and indicated that dehydration was both linear and temperature dependent. By increasing the temperature, the boundaries for the activation energy were altered resulting in greater mobility of the bound water for mass transfer. Finally, scale-up designs were investigated and reported. Once again, the issue of available conditions and options to alter equipment were limiting. For scale-up, the use of dry nitrogen sweeps, and dew-point hygrometers were investigated. Dew-point hygrometers were successful in determining the endpoint of drying without dehydrating the monohydrate. This was done by utilizing a dew-point hygrometer that could be used in the presence of IPA to measure the dew point of the dryer vapor effluent. Assuming constant air pressure and water content, the dew point is the temperature to which air must be lowered to become saturated with water vapor. When the moisture capacity decreases below the dew point, any airborne water vapor condenses to produce liquid water, or dew. In this reported case study, dew-point data was used to generate drying curves. First by converting the dew point values to partial pressures using dewpoint/frost-point tables and then by dividing the partial pressure of water by the total pressure in the dryer effluent line,

the mole fraction of water in the vapor was calculated. This gave further information into the drying optimisation where required.

The CQAs of an API, alisertib sodium, an oral oncology drug, were affected upon drying of the monohydrate, which readily dehydrated and agglomerated.^[71] From the results of small-scale investigations, it was deduced that key parameters such as pressures, nitrogen flowrate and operating temperatures majorly impacted the level of agglomeration. The authors of the study also detailed the importance of solvent composition within the crystallisation process prior to drying. A correlation between the amount of water in a wash and the level of agglomeration was clearly shown. APIs, like alisertib sodium, which are hygroscopic, are known to form agglomerates in aqueous environments due to self-hydrogen bonding. Compounded with the acicular shape of the API, this gives rise to a higher surface area for interaction. The importance of scaling-up of experiments was noted as, in this case, the scale-down setup was not representative in relation to heat and mass transfer of the drying during pilot scale experimentation. However, the key parameters outlined in the lab-scale experiments helped to define a revised drying protocol which was tested at large-scale.

Also with respect to agglomeration, literature detailed^[72] the effect of the agglomerate structure of carbamazepine dihydrate on drying and dehydration kinetics and stability. Agglomerates exceeding a certain particle size, were found to retain an excess amount of unbound, physically adsorbed water, relative to the smaller agglomerate sizes. The large agglomerates resulted in a more stable dihydrate when compared to the smaller particle size, implying the presence of unbound moisture increased the stability of the hydrate. It was discussed that the ability of the large particles to retain the unbound moisture was caused by a 'forced' hydrate

preparation method, whereby the crystals were fully submerged in water to generate the dihydrate and then hydrophobic interactions occurred inducing agglomeration. ^[73] After filtration, excess unbound moisture caused levels of cohesion between the primary particles which, upon drying, resulted in crystalline bridges forming. This then gave rise to highly dense, large particles. By exposing these large particles to varying levels of humidity, it was determined that hydrate stability was found at low environmental moisture levels, suggesting stability was enhanced by the presence of this unbound moisture. This work also demonstrates that the drying of agglomerate-forming hydrates can be manipulated so that the physical stability is increased.

From the articles discussed above, the influence of drying parameters such as temperature, pressure, humidity and even nitrogen flowrate have been clearly demonstrated. The choice of dryer was also shown to impact drying. Understanding the stability and drying kinetics associated with an API allows for an optimal drying process to be designed.

1.2. Model compounds

To examine the drying of hydrate forms, carbamazepine (CBZ) and theophylline (THEO) were chosen as model APIs for this project, due to their hydrate forms and numerous polymorphic forms.

1.2.1. Carbamazepine (CBZ)

CBZ has a molecular formula of $C_{15}H_{12}N_2O$ with a molar mass of 236.67 g/mol. Its structure is shown in **Figure 1.11**.

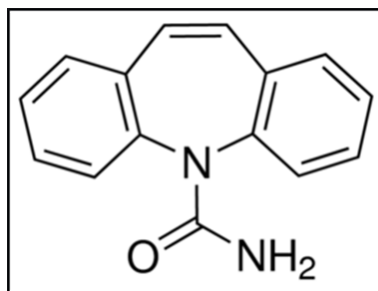


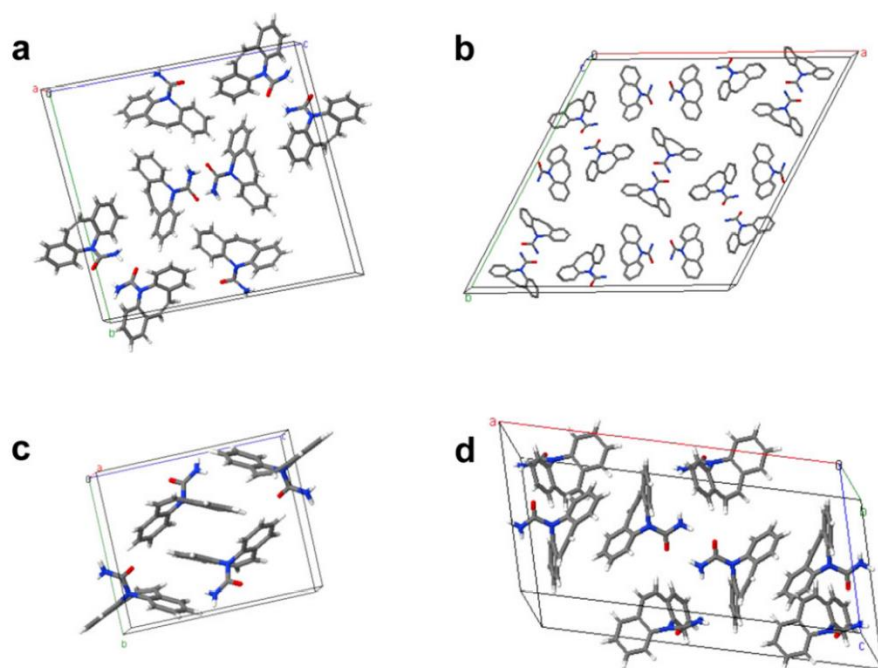
Figure 1.11: Chemical structure of anhydrous CBZ.

CBZ is an anticonvulsant and mood-stabilizing drug, commonly used in the treatment of epilepsy, trigeminal neuralgia and bipolar affective disorder. ^[74] Ion channel blocking is the mechanism of action, and it prevents persistent repeated neural excitation.

CBZ contains one hydrogen bond acceptor group and is known to form four anhydrous polymorphs and a dihydrate form. It is a well-investigated drug substance concerning polymorphism, crystal packing, solid phase stability and the resulting phase transformations. ^[31, 34, 46, 75] Of all the polymorphs, Form III a *P*-monoclinic crystal, is the only one which displays therapeutic benefits. Form I is a triclinic crystal which has an enantiotropic relationship with Form III based on thermochemical data ^[76, 77] and densities ^[78]. Studies have found that the four anhydrous polymorphs are close in energy with the stability order (at room temperature) being III > I > IV > II. ^[78] Trigonal, Form II has the lowest stability, lower than the *C*-monoclinic Form IV. Gaining thorough knowledge of the trigonal form's relationship to the other polymorphs is challenging due to its fast transitions, and certain attributes, like its melting point, are unknown. ^[31]

With respect to the crystal packing of the anhydrous polymorphs (**Figure 1.12**), each share a similar hydrogen bonding motif consisting of a dimer with two amide-amide hydrogen bonds. The

alternative packing of dimers into stable structures is the cause of polymorphism within CBZ. For Forms I and II, the primary interaction between the dimers is caused by offset π - π stacking of the aromatic rings, while for Forms III and IV, the aromatic rings in both form the previously mentioned π - π stacking and also edge-to-face contacts in an interlocked arrangement. ^[79] Crystal packing determines properties, and with different packing comes different properties, both chemical and physical, e.g., melting points, dissolution rates and bioavailability. Phase transitions between polymorphic forms can occur due to processing, mechanical stresses, and storage conditions.



[80]

Figure 1.12: Packing diagrams of CBZ's four anhydrous polymorphs: (a) Form I, (b) Form II, (c) Form III and (d) Form IV. Image taken from the literature ^[81] which is from the Cambridge Crystallographic Data Centre.

CBZ has one hydrate form, the dihydrate. Small differences in thermochemical behaviour have been noted in the dihydrate forms when generated from the Form III polymorph and the Form I ^[20], but due to XRPD and spectroscopic data being identical, they are not considered to be two distinct hydrate forms. Formation of the dihydrate can be achieved through multiple methods, such as recrystallisation from a wet ethanol solution ^[75], slurry conversion in deionised water ^[20], or by keeping a portion of the anhydrous form in a high humidity environment (>52%) ^[38]. Crystal packing, **Figure 1.13**, shows four water molecules forming a channel along the c-axis in each layer of the hydrated form, allowing water molecules to easily escape under dehydrating conditions. ^[31] The dihydrate form is stable at room temperature and at humidities higher than 52% RH but is basically insoluble in water ^[82], with little use as a therapeutic in comparison to the Form III anhydrous polymorph. Spontaneous hydrate formation has previously been discussed, and CBZ is a good example of when it should be avoided and why storage conditions are important. As with many hydrated APIs, changes in relative humidity, pressure and/or temperature can cause dehydration or transformation to an anhydrous polymorph. ^[38, 80] A transformation back to the Form III polymorph is known to occur at high temperatures or pressures. ^[80] With respect to the drying of CBZ dihydrate, which is ultimately one of the main aims of this project, the use of high water vapour pressures levels are known to preserve the hydrated form. ^[38, 83] Mechanical stresses, like milling and grinding, are also known to alter the dehydration mechanism. Dehydration of the hydrated form leads to transitions to different forms. The anhydrous Form I is generated by heating the dihydrate, while Form III can be obtained when dehydration with reduced pressure is utilised. When dehydration takes place under a nitrogen sweep or under

low pressure, this results in the amorphous form being generated.

[20]

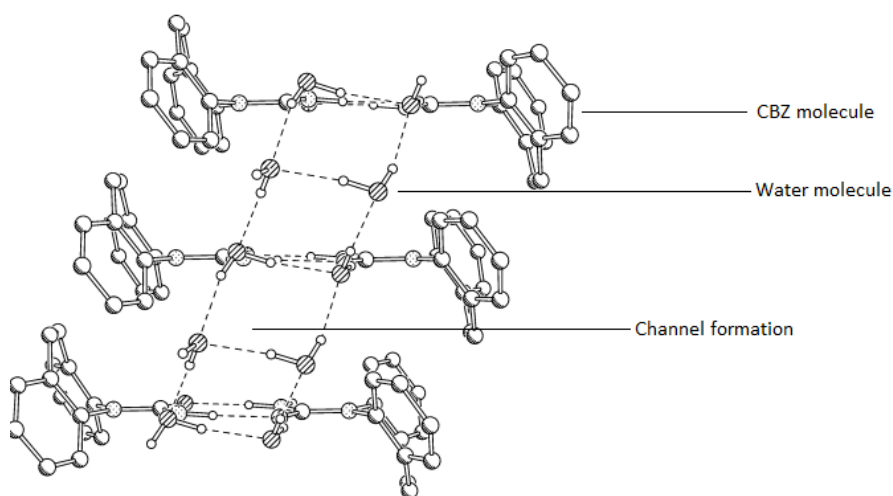


Figure 1.13: CBZ dihydrate's hydrogen-bonded network, viewed down the a axis. Adapted from literature. ^[31]

1.2.2. Theophylline (THEO)

THEO has a molecular formula of $C_7H_8N_4O_2$ with a molar mass of 180.16 g/mol. Its structure is shown in **Figure 1.14**. THEO is a bronchodilator which is used to treat asthma. ^[84] It has recently been demonstrated that, at lower concentrations, it has anti-inflammatory benefits on asthma and COPD. Phosphodiesterase(PDE)3 inhibition is the mechanism of action for bronchodilatation, however, the suppression of PDE4 and histone deacetylase(HDAC)-2 activation, which results in the switching off of active inflammatory genes, may also be a cause of the anti-inflammatory action.

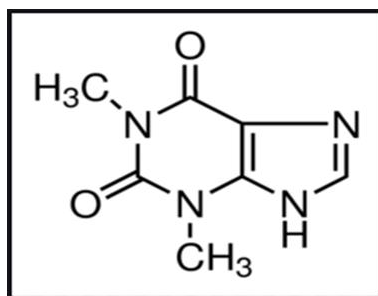


Figure 1.14: Chemical structure of anhydrous THEO.

THEO has been found to exist as four anhydrous polymorphs ^[85, 86] and a monohydrate. ^[87] Form II is the commercially used polymorph which exhibits the therapeutic benefits. It is also the stable form at room temperature. ^[88] This form is produced through the dehydration of the monohydrate and has a orthorhombic structure. ^[84] An enantiotropic relationship is evident between Form II and Form I, with the latter being stable at higher temperature. ^[84] Form I may also be generated through the dehydration of the monohydrate form. ^[89] Form III is a highly metastable form which readily undergoes conversion back to form II. ^[84] It can be produced by dehydration ^[90] or vacuum drying ^[86] of the monohydrate. The final anhydrous form (Form IV) can be generated through a solvent-mediated process from either Form I or II, and is considered the most thermodynamically stable of the anhydrous forms. ^[86] It is monoclinic in structure and differs greatly to the other polymorphs which have hexagonal plate-like morphologies. The stability order of these forms has been noted as: Form IV > Form II > Form I > Form III. As with other APIs, different polymorphic forms have different properties and theophylline is no different. The generation of a metastable form post dehydration for the hydrated form, followed by its conversion to Form II has been found to increase tablet hardness and cause the failure of dissolution rate testing of theophylline tablets. ^[90]

Figure 1.15 shows the crystal packing of both the anhydrous Form II, commercially available polymorph, and the monohydrate form of THEO. In contrast to the dimers in the monohydrate, the packing for the anhydrous forms is made up of hydrogen bonds between N-H $\cdots$ N and two bifurcated hydrogen bonds between C-H $\cdots$ O, giving a bilayer structure. ^[86]

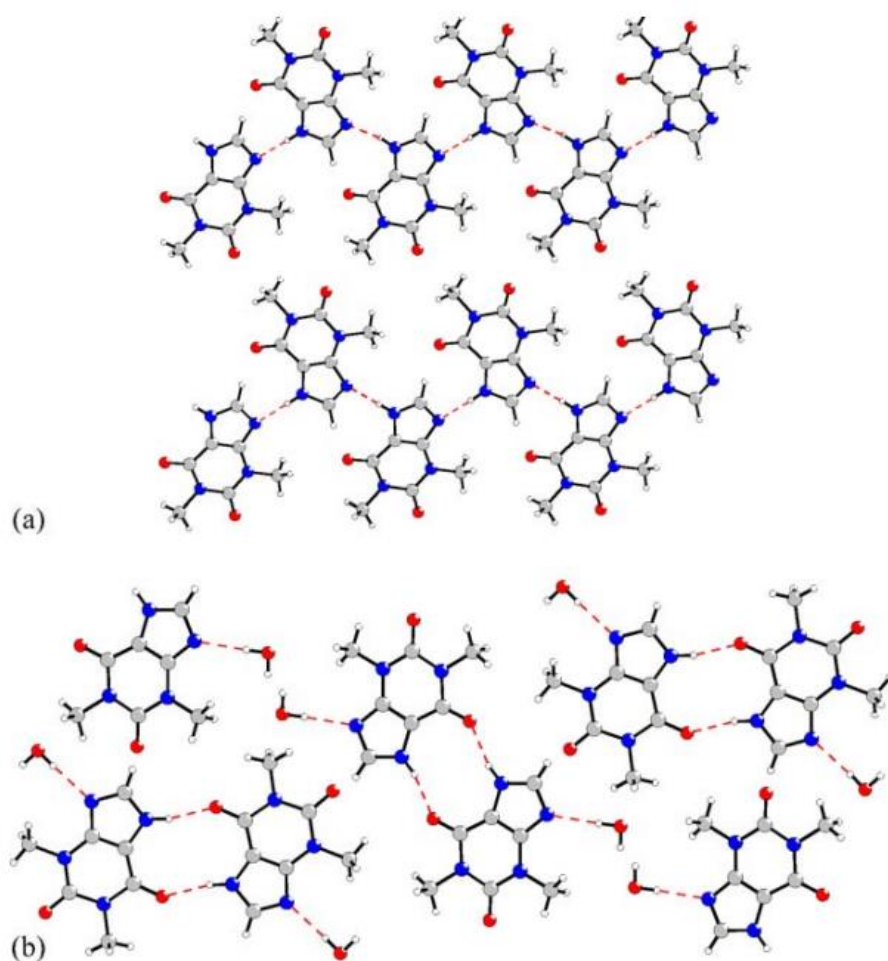


Figure 1.15: Packing diagram of (a) anhydrous theophylline ^[91] and (b) theophylline monohydrate. ^[87] Image taken from literature. ^[92]

THEO monohydrate is a monoclinic, channel hydrate with THEO molecules hydrogen bonding to form dimers, with water molecules forming cross linked parallel chains with said dimers by more hydrogen bonds, to create layers. ^[87] The hydrated form is

generated through cooling crystallisation experiments ^[86] or when anhydrous Form I is exposed to high humidities. ^[88]

The impact of numerous factors, including temperature, ^[93] particle size, ^[94] grinding, ^[95] and tableting, ^[96] on THEO monohydrate's tendency to become dehydrated has been studied. As a channel hydrate, dehydration temperatures tend to be lower than that of other hydrates (isolated-site or ion coordinated hydrates) and with predetermined pathways for exit already in place, dehydration tends to take place readily with the lattice remaining intact. While the monohydrate has better tableting and mechanical properties, such as better compactability and lower elasticity, compared to anhydrous Form II, the compression of hydrated crystals was found to induce dehydration and transition to the metastable form, followed by a second transition to Form II. ^[90]

1.3. Project Aims

The main aim of this project is to study the impact of drying conditions on the stability of the API hydrate form and to develop a drying platform which can consistently produce the desired, dry hydrate form.

Some central research questions that will be answered through this work include:

- How is hydrate stability/dehydration affected by micromeritic properties, like particle size?
- What parameters influence the drying of hydrated APIs?
- Can excess, loosely bound moisture be removed without affecting the water of hydration? If so, can the time for drying to this point be predicted?

1.4. Project Roadmap

The structure of this project can be broken down into seven sections, shown in **Figure 1.16** . Firstly, a literature review was carried out to provide context, gather current industry understanding, select model compounds, determine well-established methodologies for the generation and characterisation of hydrate and polymorphic forms of said compounds, determine the gaps in knowledge, and to further understand the significance of this work.

Once completed, lab work began with the generation and characterisation of the hydrate forms and their associated polymorphic forms to help build a library of results to facilitate future form identification purposes. Solubility data was then gathered to inform other methods for the generation of the hydrate forms with differing PSDs. Having samples of the hydrate form with differing PSDs allows for the effects of micromeritic properties to be investigated.

Once robust methods of hydrate generation were confirmed, a hydrate stability study was undertaken whereby portions of dry hydrate crystals of increasing PSD were placed in controlled temperature and % RH environments. Through this study, a greater insight into the design space where hydrate stability could be found, and for how long, was determined. Information from this work allowed for future drying studies to be data driven and to come from a place of knowledge.

From the hydrate stability study, the need for % RH control proved integral, so for a drying study, having access to highly controlled % RH was focused on. Through several knowledge share sessions about lab equipment, what was available and what conditions could be achieved, the DVS Intrinsic became the instrument of

interest. With it, a small-scale drying study was undertaken where drying kinetics data was collected, examining the effects of temperature, % RH, PSD and cake depth on the drying, hydrate stability and the dehydration of the samples.

DVS data generated was then coupled with information from the literature to build a convective drying model which was intended to predict the drying time to produce the dry hydrate form of the model hydrated APIs. A scaled-up drying study was carried out to test the model.

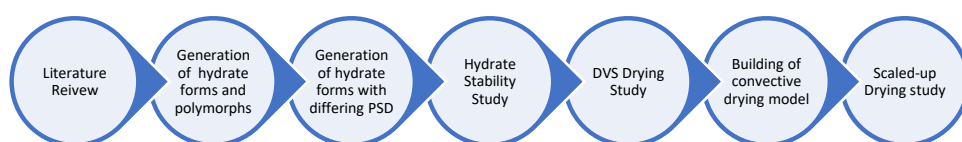


Figure 1.16: Project roadmap.

CHAPTER 2. Materials and Methods

2.1. Materials

Table 1.1: List of materials and suppliers used

Chemical	Supplier
Carbamazepine (CBZ) anhydrous	Sigma Aldrich (Ireland)
Theophylline (THEO) anhydrous	Sigma Aldrich (Ireland)
Heptane (> 99%)	Sigma Aldrich (Ireland)
2-Propanol (> 99%)	Sigma Aldrich (Ireland)
Ethanol (> 99%)	Fischer Scientific (Ireland)
Acetone (ACS Grade)	Sigma Aldrich (Ireland)
Methanol (> 99%)	Sigma Aldrich (Ireland)
Deionised water (DIW)	arium® Comfort II Combined Lab Water System bench-top, Sartorius system - delivering Type 1 ultrapure water
Span 80	Sigma Aldrich (Ireland)
Hydranal [™] , Water Standard, 10.0 mg/g	Honeywell

2.2. Methods

2.2.1. Solubility

2.2.1.1. Solubility data for CBZ dihydrate via the gravimetric method

Due to CBZ's low solubility in water, the gravimetric technique for solubility determination was used. In this method the dissolved concentration of CBZ dihydrate in deionised water (DIW) at 20, 40, 60 and 80 °C was measured after being held for 24 hr. Measurements were made for each temperature in triplicate using three 25 mL EasyMax™ (EM) tubes which sit within a sleeve and then within the jacketed EM™ workstation. Approximately 300 mg of CBZ dihydrate was added to 10 mL of DIW and stirred over 24 hr at 250 rpm using a magnetic stirrer. Temperature was controlled by the EM™ software (iControl 6.0) with the temperature set at 3 °C higher than the target temperature for each experiment to account for the heat transfer through the sleeve. After 24 hr, using a glass pipette, 1 mL of the solution was extracted from the crystallizer tube and transferred into a 2 mL syringe. A syringe filter (polytetrafluoroethylene (PTFE), pore size 0.2 µm) was used to filter the solids present in the solution and the clear liquor was transferred into a previously weighed glass vial. The glass pipettes, syringes, and syringe filters were all kept in a drying oven overnight to ensure accuracy and prevent precipitation prior to or during filtering. The glass vials with clear liquid solution were placed at the back of a fumehood to allow for the water to evaporate. The mass of the sample before and after drying were measured using an electronic analytical balance (PA214, OHAUS, linearity of ±0.3 mg). The concentration of CBZ dihydrate is determined as:

$$C \left(\frac{\text{g}}{\text{kg}} \text{ solvent} \right) = \frac{\text{mass of solid residue (g)}}{\text{mass of solution (g)} - \text{mass of solid residue (g)}} \times 1000$$

Eq. 1

Solid samples at the end of the experiment were then analysed by DSC (see **Section 2.4.1** for sample preparation) to confirm the presence of the dihydrate.

2.2.1.2. Solubility data for THEO Monohydrate using the Crystal 16™ equipment

A Technobis Crystal16™ with CrystalClear™ software was used to gather solubility data for THEO monohydrate. A Crystal16™ was used to pin-point the temperatures at which a suspension, at a specific concentration, becomes a clear solution while using a heating ramp. It combines automation with integrated turbidity measurements to read when light can go through a vial uninterrupted, giving 100% transmissivity (**Figure 2.1**).

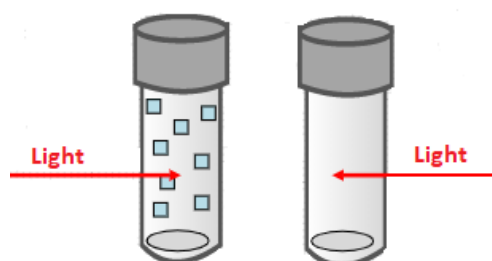


Figure 2.1 Schematic of Crystal16™ measurement process

The Crystal16™ operating conditions used are outlined in

Table 1.2. 100 mg of previously generated THEO monohydrate (see **Section 2.3.1.6**) was accurately weighed into a Crystal16™ vial. 1 mL of DIW was charged using a micropipette and a magnetic stirrer

was added to the vial. The mixture was stirred at 250 rpm and heated at 0.1 K/min from 20 °C to 70 °C. The clear point was determined when the transmittance reached 100%. The analysis was undertaken in triplicate.

Table 1.2: Crystal16™ parameters used for THEO monohydrate samples.

Parameters	
Heating/cooling type	Peltier
Measurement type	Turbidity
Temperature accuracy	0.1 K
Heating/cooling rate	0.1 K/min
Stirrer speed	250 rpm

2.3. Unit Operations

2.3.1. Crystallisation

2.3.1.1. Equipment set-up - Mettler Toledo EasyMax™ System

A Mettler Toledo EasyMax™ (EM) workstation is equipped with two 100 mL glass reactors, allowing for two experiments to be conducted in parallel. Experimental volumes have a range of 20 to 100 mL, or alternatively, four 25 mL test tubes. Heating/cooling rates, reactor temperature, agitation (rpm) and automated dosing are controlled using the associated touchpad or using the system software, iControl 6.0 (Mettler Toledo). The jacket temperature can be changed at a rate up to 10 °C/min. In crystallisation experiments, agitation was provided by using a steel pitch blade impeller of 38 mm diameter and 45° inclined blades for all experiments. The vessels of this system are equipped with sample ports that allow for experiments to be coupled with PAT tools.



Figure 2.2 Mettler Toledo EasyMax™ Workstation

2.3.1.2. Preparation of carbamazepine (CBZ) dihydrate

CBZ dihydrate was prepared by suspending anhydrous Form III CBZ in deionised water (DIW) and stirring for a minimum of 24 hrs. The conversion was completed in the EM System, using a glass 100 mL crystalliser vessel where agitation was maintained at 150 rpm. DIW was added to the vessel and maintained at 25 °C. Commercial anhydrous CBZ was charged in at a concentration of 66 mg/mL. The slurry was stirred for a minimum 24 hr as reported in literature. ^[20] The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 µm. Solids were then dried ambiently, in the fumehood overnight. PAT such as the Particle Vision and Measurement (PVM) were employed to further investigate the conversion to the hydrated form (see **Section 2.6.2**).

2.3.1.3. Preparation of CBZ dihydrate of differing sizes

2.3.1.3.1. Preparation of Small CBZ dihydrate crystals

Approximately 1 g of previously generated CBZ dihydrate (see **Section 2.3.1.2**) was re-slurried in 100 mL of heptane. An IKA T10 basic homogenizer work centre (handheld mill) was used, and the suspension was milled on the highest setting for 30 sec intervals, with 30 sec breaks, for a total of 5 min. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 μm . Solids were then dried ambiently, in the fumehood overnight. Downsizing of particle size was confirmed using off-line particle size distribution analysis (see **Section 2.4.6**).

2.3.1.3.2. Preparation of Medium CBZ dihydrate crystals

Crystals generated from the standard method for the dihydrate were considered 'Medium' in size. See **Section 2.3.1.2**.

2.3.1.3.3. Preparation of Large CBZ dihydrate crystals

Anhydrous Form III CBZ was stirred at 25 °C and 150 rpm for 3 hr. The solution was then heated to 70 °C at 2 °C/min and held for 1 hr. A cooling rate of 0.1 °C/min reduced the temperature to 25 °C. The heating and cooling steps were repeated three times. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 μm . Solids were then dried ambiently, in the fumehood overnight. Confirmation of crystal growth was done using PSD analysis (see **Section 2.4.6**)

2.3.1.4. Preparation of 'wet' CBZ dihydrate crystals

CBZ dihydrate crystals were generated via the standard method detailed in **Section 2.3.1.2**. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 μm . Filtration was staggered. Vacuum was applied initially for 3 min and then turned off. A small portion of sample was taken for analysis by the Moisture Analyzer (**Section 2.5.2**) to determine % total Moisture Content. During this time, the vacuum remained off and tinfoil was placed over the Buchner funnel to prevent any further loss of moisture. If the sample had a % total Moisture Content of 30-45%, the sample was considered 'wet' enough for drying via the DVS (**Section 2.8.2**) or in scaled-up oven drying (**Section 2.10**). If too 'wet', vacuum was applied for an additional 2 min and the analysis was repeated. 5-7 min under vacuum consistently gave a sample of CBZ dihydrate with appropriate % total Moisture Content levels.

2.3.1.5. Preparation of carbamazepine polymorphs

2.3.1.5.1. Form I

Approximately 0.5 g of commercial anhydrous CBZ was charged into a glass 100 mL vessel and placed into the EM System. Agitation was set to 200 rpm and the reactor temperature (T_r) was set to 140 °C and held for 5 hr. This method was reported in literature.^[76] The resulting solid was allowed to come to room temperature overnight prior to characterisation.

2.3.1.5.2. Form II

Based on a method reported in literature,^[78] anhydrous CBZ Form II was crystallised from anhydrous Form III CBZ using the EM System

with a glass 100 mL vessel and EtOH as the solvent. 40 mL of EtOH was heated to 80 °C over 20 min and maintained at 150 rpm. Once at temperature, 1.9264 g of anhydrous Form III CBZ was added. The solution was held at 80 °C for 30min. It was then slowly cooled at 0.1 °C/min to 20 °C and held for 30 min. The final cooling step involved further cooling to 5 °C over 5 hr. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 µm. The solids were then dried under reduced pressure in a vacuum oven.

2.3.1.5.3. Form IV

A method in literature detailed ^[97], approximately 0.5 g of previously generated CBZ dihydrate (**Section 2.3.1.2**) was placed in a Cuspor vial along with a 2% RH dessicant capsule (see **Figure 2.4** for setup) and placed into a vacuum oven (Binder VDL 23) at 20 °C for 24 hr.

2.3.1.6. Preparation of theophylline (THEO) monohydrate

THEO monohydrate was prepared via a cooling crystallisation. The EM System was used with a glass 100 mL vessel. DIW was charged into the vessel and brought to 85 °C with agitation being maintained at 200 rpm. Once at temperature, anhydrous Form II THEO was added at a concentration of 125 mg/mL, as previously reported in literature. ^[86] The solution was then held for 15 min. A cooling rate of 0.3 °C/min was used to bring the solution to 10 °C. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 µm. Solids were then dried ambiently, in the fumehood overnight. A combination of in-situ tools such as Focused Beam Reflectance Measurement (FBRM) (see **Section 2.6.1**) and PVM

(see **Section 2.6.2**) were employed to further investigate the conversion to the hydrated form.

2.3.1.7. Preparation of THEO monohydrate of differing sizes

2.3.1.7.1. Preparation of Small THEO monohydrate crystals

Using the EM System, DIW was charged into a 100 mL vessel and brought to 85 °C with agitation at 200 rpm. Anhydrous Form II THEO was added at a concentration of 125 mg/mL. The solution was then held for 15 min. A cooling rate of 1 °C /min was used to reduce the temperature to 10 °C for Small crystals. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 µm. Solids were then dried ambiently, in the fumehood overnight.

2.3.1.7.2. Preparation of Medium THEO monohydrate crystals

Crystals generated from the standard method for the monohydrate were considered 'Medium' in size. See **Section**

2.3.1.6.

2.3.1.7.3. Preparation of Large THEO monohydrate crystals

Using the EM System, DIW was charged into a 100 mL vessel and brought to 85 °C with agitation at 200 rpm. Anhydrous Form II THEO was added at a concentration of 125 mg/mL. The solution was then held for 15 min. A cooling rate of 0.1 °C/min was used to reduce the temperature to 10 °C for Large crystals. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 µm. Solids were then dried ambiently, in the fumehood overnight.

2.3.1.8. Preparation of 'wet' THEO monohydrate crystals

THEO monohydrate crystals were generated via the standard method detailed in **Section 2.3.1.6**. The solid was isolated by vacuum filtration, using house vacuum, a Buchner funnel and Whatman filter paper, 70 mm, with a pore size of 0.45 μm . Filtration was staggered. Vacuum was applied initially for 3 min and then turned off. A small portion of sample was taken for analysis by the Moisture Analyzer (**Section 2.5.2**) to determine % total Moisture Content. During this time, the vacuum remained off and tinfoil was placed over the Buchner funnel to prevent any further loss of moisture. If the sample had a % total Moisture Content of 30-45%, the sample was considered 'wet' enough for drying via DVS (**Section 2.8.2**) or in scaled-up oven drying (**Section 2.10**). If too 'wet', vacuum was applied for an additional 2 min and the analysis was repeated. 3-5 min under vacuum consistently gave a sample of THEO monohydrate with appropriate % total Moisture Content levels.

2.3.1.9. Preparation of theophylline polymorphs

2.3.1.9.1. Form I

Based on literature, ^[89] a portion of previously generated monohydrate (**Section 2.3.1.6**) was heated to 100 °C for 24 hr. This was completed using the Crystalline system, which is a benchtop parallel crystalliser, commonly used for gathering crystallisation and formulation data. The instrument used was the Standard Edition (SE) which can hold 8 standard glass vials (16.6 mm diameter, flat bottomed and 8 mL volume) where the temperature conditions can be controlled for each vial. 0.5 g of previously generated monohydrate was placed into a Crystalline vial with an overhead stir shaft, see **Figure 2.3**. The vial was then placed into a

Crystalline system. The temperature was set to 105 °C, with the extra 5 °C used to ensure that solid temperature reaches 100 °C. Speed for the impeller was set to 50 rpm, to help aid heat transfer but not too high that breakage of crystals would become an issue. After 24 hr, the sample was removed and bottled.



Figure 2.3: Crystalline vial with overhead impeller.

2.3.1.9.2. Form III

To generate Form II, a method from literature ^[85] was used which involved the dehydration of previously generated monohydrate (**Section 2.3.1.6**) crystals under reduced pressures. The method detailed involved a portion of monohydrate being placed into a vacuum oven at 15 °C and (<0.05 Torr). For this a portion of monohydrate being placed onto a plastic weigh boat and covered with 1 ply of tissue paper (to prevent sample movement during vacuum release) and allowed to remain at the specified conditions for 24 hr.

2.3.1.9.3. Form IV

Based on literature, ^[86] Form IV was prepared by suspending excess anhydrous Form II THEO in a methanol (MeOH)-water mixture, whereby the volumes of MeOH and water used achieved a water

activity value of <0.68 . The suspension was then stirred and until a morphological change from needles to plate like crystals was seen. Microscope samples were taken to track these changes over time. The literature on this stated this conversion occurred by day four. Using an in-house water activity calculations template, it was found that mixing 34.5 mL of water with 65.5 mL of MeOH gave a water activity value of 0.63 when at 25 °C. 100 mL of the MeOH/water mixture was placed into a 250 mL Duran, along with a magnetic stir bar. To achieve the saturated solution, 2.4166 g of anhydrous Form II THEO was added. The Duran was sealed with a screw cap and placed on a stir plate with a level 1 rpm value. The solution was allowed to stir at room temperature until microscope images showed the conversion had taken place.

2.4. Solid state characterisation

2.4.1. Differential Scanning Calorimetry (DSC)

Analysis was carried out using a Netzsch DSC 200F3 Maia with nitrogen flow, autosampler, reference loaded furnace and triple pin-holed aluminium crucibles. Netzsch-Proteus™ 6.1.0 software was employed for data analysis. Calibration was completed using an indium standard and its thermograph was then compared with those provided by the vendor. The weighing platform and empty crucible was placed on the balance and tared. 2-5mg of the sample was added. The crucible was non-hermetically sealed and placed into the autosampler. For theophylline samples, pans were pierced (three pin holes), sealed non-hermetically and analysis was carried out from room temperature to 300 °C at 10 °C/min. The Form I polymorph had a melt temperature of ~ 290 °C which dictated the endpoint temperature. For carbamazepine samples, analysis was carried out from room temperature to 235 °C at 10 °C/min. A lower endpoint temperature was used as the CBZ Form I polymorph had

a melting point at ~ 190 °C. For form identification purposes, when generating batches of hydrated crystals or the batches of each polymorph, these samples were carried out singly. For drying studies via DVS, DSC analysis of pre and post analysis was carried out in triplicate. For the scaled-up oven drying experiments, samples were done in duplicate.

2.4.2. Thermogravimetric Analysis (TGA)

A Thermal Advantage Q50 TGA was used in conjunction with Thermal Advantage Release 5.4.0 software to analyse samples. The TGA sample holder with an empty DSC pan was first tared within the instrument. Sample (~ 5 -15mg) was then packed into the pan as carefully as possible, ensuring a tight packing so that portions did not fall off entering the instrument or during the analysis. All samples were analysed by heating from room temperature to 300 °C at a heating rate of 10 °C/min under a nitrogen flow of 60 mL/min over the sample. For form identification purposes, when generating batches of hydrated crystals or the batches of each polymorph, these samples were carried out singly. For pre drying study samples, of 'wet' hydrated crystals, TGA analysis was carried out in duplicate. TGA was used on the 'wet' samples to provide information on the levels of bound and unbound moisture. For drying studies via the DVS, post drying samples were analysed singly due to the small sample size. Where TGA analysis was needed for the scaled-up oven drying experiments, analysis was carried out singularly.

2.4.3. Dynamic Vapour Sorption (DVS)

A DVS Intrinsic (Surface Measurement Systems, London, UK) was used for all experiments. The temperature range of the instrument is 20-40 °C. The accuracy of the system is ± 0.2 °C for temperature

and ± 1.0 °C for the relative humidity. Initial experiments to examine sorption/desorption behaviour of the crystals, a $\frac{dm}{dt}$ method was used. Approximately, 15-25 mg of the sample was loaded onto a metal sample pan. The first stage involved drying the sample at 0% RH and 25 °C for 10 hr. Thereafter, the sample underwent 10% RH increases up to 90% RH and the time taken for each increase was dependent on the sample weight reaching equilibrium, whereby the change in mass was ≤ 0.002 mg/min. Dehydration followed similar increments from 90% RH back to 0% RH. For drying studies using the DVS, more details can be found in **Section 2.8**. For hydrate confirmation purposes, when generating batches of hydrated crystals, these samples were analysed singly. For the small-scale drying studies using the DVS, analysis was carried out in triplicate for each set of conditions.

2.4.4. X-Ray Powder Diffraction (XRPD) - Brucker

A Brucker D2 PHASER Diffractometer was used with DIFFRAC.SUITE software for analysis. CBZ samples were analysed by scanning from 3 – 40 °, with a step size of 0.05° 2 θ at 1 sec/step using Cu K α radiation. Voltage and current were 30 kV and 10 mA, respectively. THEO samples were analysed by scanning from 5 – 40 °, with a step size of 0.01° 2 θ at 1 sec/step using Cu K α radiation. All samples were analysed in airtight sample holders, as this is the practice used within the lab. The sample was carefully placed into a Bruker steel sample base and gently compressed into the holder to give a uniform surface. A small piece of Kapton foil is placed across the sample, an O ring and top half of the sample holder is placed on top, sealing the sample. Up to six samples can be placed into the auto-sampler. For form identification purposes, when generating batches of hydrated crystals or the batches of each polymorph, these samples were analysed singly.

2.4.5. XRPD - Malvern PanAnalytical Empyrean

A second XRPD was also used in later work (Drying Studies via DVS and Scale-up oven drying experiments). This was a Malvern PanAnalytical Empyrean Series 3 Power X-ray Diffractometer with Data Collector (version 7.1) software. Data analysis was completed using Data Viewer (version 1.9a) software. Samples were analysed by scanning from 5 – 45 °, with a step size of 0.01° 2θ at 1 sec/step using transmission mode and Cu Kα radiation. Voltage and current were 45 kV and 40 mA, respectively. All samples were analysed in airtight sample holders with Kapton foil. For form identification purposes, when generating batches of pre-drying studies samples, of ‘wet’ hydrated crystals, post DVS samples and for the scaled-up oven drying experiments, analysis was carried out singularly.

2.4.5.1. Non-ambient Chamber

The non-ambient chamber is an attachment used within the previously mentioned XRPD instrument. It was an Anton-Paar MHC-Tran with TCU 60 M and MHG 32 controllers. The MHC-trans was designed for powder samples. There are two modes of operation available:

1. Humidity mode

Total temperature range: +10 to +120 °C

Full humidity control range: 4% RH to 85% RH at +10 °C

4% RH to 95% RH between +20 °C to 60 °C

4% RH to 70% RH up to +80 °C

Available gases: Air, nitrogen

Gas flow rate: 50 mL/min to 250 mL/min

2. Temperature mode

Total temperature range: -10 °C to +150 °C

Available gases: Air, nitrogen

Gas flow rate: 0 mL/min to 100 mL/min

Samples were analysed by scanning from 5 – 50 °, with a step size of 0.01° 2 θ at 18.9 sec/step using transmission mode and Cu K α radiation. Voltage and current were 45 kV and 40 mA, respectively. All samples were analysed in chamber specific holders. Data analysis was completed using Data Viewer (version 1.9a) software.

2.4.6. Particle Size Distribution (PSD) Analysis

A Malvern Mastersizer MS3000 with the Hydro MV dispersion unit was used with Mastersizer 3000 Version 3.6 software for the analysis. Parameters used are shown in **Table 1.3** below.

Table 1.3: Malvern Mastersizer 3000 parameters for sample measurements

Parameters	
Particle type/optical model	Fraunhofer
Analysis model	General purpose
Particle shape	Non-spherical
Sample measurement time	45 sec
Background measurement time	30 sec
Obscuration limits	5-20%
Dispersant	Heptane 0.2% v/v SPAN80
Dispersant RI	1.39
Number of measurements per aliquot	3

Analysis sensitivity	Normal
Pump speed	2500 rpm
Sonication	No

Samples were added to the dispersion unit by direct addition. A spatula tip at a time was added until obscuration limits were reached. For theophylline samples were stirred for 1 min to stabilised prior to measurement. For carbamazepine, samples were stirred for 10 min to allow for deagglomeration and stabilisation prior to measurement.

At a minimum, all samples were analysed in duplicate and where sample amounts allowed analysis was carried out in triplicate. For post DVS samples due to the small sample size (10-20 mg), single analysis was completed. To maximise data from the limited sample amount, a small volume wet sample dispersion unit was used on the Malvern 3000. The Hydro SV is a unit which enables analysis on using small volumes of sample and dispersant. The cell holds a volume of 5.6 – 7 mL of dispersant and contains a software controlled magnetic stirrer for dispersion control. Sample retention is also possible which is advantageous.

Results were then presented using a cumulative PSD distribution graph along with details of specific particles sizes under the headings of percentile values; d10, d50 and d90. As an example, if the d90 value is 100nm, this means that 90% of the sample has a particle size of 100nm or less.

2.4.6.1. Methodology for determining statistical differences of data

A t-test is a statistical test used to determine if there is a significant difference between the means of two groups or samples. It is commonly used when the data follows a normal distribution and the variances of the two groups are assumed to be equal.

The t-test compares the means of the two groups and calculates a t-value, which represents the difference between the means relative to the variability within the groups. The larger the t-value, the more likely there is a significant difference between the means.

The t-value is calculated using the formula:

$$t = (\text{Mean}_1 - \text{Mean}_2) / (s / \sqrt{n})$$

where Mean_1 and Mean_2 are the means of the two groups, s is the pooled standard deviation, and n is the sample size. The pooled standard deviation combines the variability within each group to estimate the overall variability. Once the t-value is calculated, it is compared to the critical values. The critical values depend on the desired significance level which was set to 0.05. If the calculated t-value exceeds the critical value, there is evidence to reject the null hypothesis and conclude that there is a significant difference between the means of the two groups.

2.4.7. Light Microscopy

Images of samples were taken using an Olympus BH-2 microscope with Pax-Cam and Pax-it! 2 Version 1.2.0.0 software. Upper and lower illumination was provided by a LED light source. A small amount of each dried sample was shaken across slides, ensuring samples were mixed to be representative. For slurries, a few drops were added to a slide and then allowed to dry. Each slide was then examined under the microscope at magnifications of x10, x20 and where possible x40 and x100, with photomicrographs acquired using the attached camera. Single samples were taken for this analysis.

2.4.8. Scanning Electron Microscopy (SEM)

A Hitachi TM 4000 Plus in conjunction with a Quorum, SC7620 sputterer was used. Voltage was 15 KV and backscattered electrons (BSE) detection was used for all samples. For samples outside drying studies using the DVS, the normal high vacuum setting was used. Pre and post drying study samples utilized the low vacuum setting on the SEM to minimise the disturbance of excess moisture in the pre, 'wet' samples. For sample preparation, an SEM stub is covered with an adhesive carbon tab and a portion of each sample was added to a HPLC glass vial. The stub is placed on top of the vial, held in contact, and shaken to allow sample to stick to stub in a concentrated area. Excess sample is tapped off. All samples are sputtered with silver-palladium alloy over 75 s under vacuum. SEM analysis was carried out singly on samples of generated polymorphs, hydrates and on the original post DVS samples.

2.5. Additional Analyses

2.5.1. Karl Fischer Analysis (KF)

A SI Analytics Titrator TitroLine® 7000 using a Hydranal™ Composite 5 setup was used for Karl Fischer titrations. The system was calibrated on each day of use, using a Hydranal™ 1% water standard achieving a % Relative Standard Deviation (RSD) <2%. 100-150 mg sample was added for analysis. Analysis was carried out in duplicate.

2.5.2. Moisture Analyzer

A Mettler Toledo Moisture Analyzer Excellence HS153 was used for % total moisture content based on loss on drying (LOD) of a small-scale sample. The system is equipped with a balance that has a max weight of 150 g and a readability of 0.1 g. Samples of approximately

0.5-2.0 g were placed onto a tared foil dish and placed into the machine for high-speed drying. Both CBZ and THEO samples were analysed with a drying temperature of 105 °C and a switch-off criterion of 3 (1 mg/50 sec). This switch-off criterion ensures that measurements are finished in the same condition (weight loss per unit time). Meaning it will look for three separate measurements where the weight changes <1 mg over a 50 sec period. The Moisture Analyzer was used for both endpoint determination of % total Moisture Content and for time point samples during some scaled-up oven drying experiment. For pre oven drying experiments, a single sample was taken to inform % Moisture Content (% M.C.) levels for inputting into the model whereas endpoint samples had duplicate analysis take place. For time point samples, these were carried out singly as it was only to give an indication of moisture levels.

2.6. Process Analytical Techniques

2.6.1. Focused Beam Reflectance Measurement (FBRM)

Mettler Toledo Particle Track S400 and G400 series were used with iC-FBRM™ Version 5.4 software. Prior to starting experiments, purging is always required, to avoid condensation inside the probe. On start-up, the air supply is set to 1 bar and 1.5 L/min for 10 min, then reduced to 0.15 L/min after that. Measurements are only taken after the probe has come to ambient conditions, this takes 30-120 sec. The probe is secured within the vessel lid, using the correct threaded holder. The optical cable and probe are then clamped for stability and safety. Via the software, the probe window cleanliness was confirmed, with total count measurements not exceeding 300 counts/s. Typically, measurement duration was set to one day and measurement intervals set at 30 s. Measurement duration was changed to 48 hrs,

should the experiment be longer. Measurement interval remained unchanged.

2.6.2. Particle Vision and Measurement (PVM)

ParticleView V19 was used with the iC PVM™ software (version 7.0.166) and the EasyViewer 400 was used with iC Vision™ software (version 8.2) was used. Each probe was inserted into the reactor using the appropriate threaded holder. Probes were left to equilibrate for a minimum of 1 hr prior to measurements. The EasyViewer 400 was used for examining the CBZ to CBZ dihydrate transition with the iC Vision™ software. This is the newest model of PVM which offered auto-focussing throughout the experiment. This proved very useful for this transition. Changes in particle shape and size were difficult to focus on with the V19 model. Measurement duration and interval was set to 1 day and 10 sec respectively. ParticleView V19 was used in all other instances of PVM usage. Measurement duration and intervals were modified where required, to cover the length of the experiment or a timepoint of interest within an experiment, such as a form transition. Due to a max number of samples being allowed to be collected within an experiment, where experiment durations were between 8-12 hr long, the measurement interval was set to 10 sec. For durations of >24 hr, the interval was set to 30 sec. Experiments were stopped and data saved when desired. For theophylline samples, the focus position ranged from 500 to 840um and for carbamazepine samples it ranged from 100-300 um. This changed based on the size of the particles being seen by the probe at the start of each experiment, focus was changed to see the clearest images.

2.7. Stability Study

2.7.1. Sample preparation of the initial stability experiment

Both the CBZ dihydrate and THEO monohydrate samples were prepared using methods detailed in **Section 2.3.1.2** and **Section 2.3.1.6**, respectively. Approximately 2 g of each sample was added to separate crystallisation dishes. One ply of tissue was placed over the top and secured in place using adhesive tape. This setup acted as a membrane, through which the sample could be exposed, and moisture could leave the sample while also minimising the possibility of cross-contamination during the experiment. Once prepared, samples were placed in a Perspex desiccator cabinet which contained a sample of phosphorous pentoxide (P_2O_5) which gave a % RH of 0% and was prepared and sealed in the same way as the samples. The cabinet containing the desiccant and samples was then placed into an IMC 18 Heratherm [™] at the desired temperature.

2.7.2. Cuspor Ageing System setup

The Cuspor Ageing System is a closed system which allows for the generation and accurate measurement of environmental conditions for the storage of small quantities of material. Temperatures of 25, 50 and 80 °C and % RH conditions of 2, 40 and 75% RH were used. **Figure 2.4** shows the equipment setup. The desired temperature environment was provided using incubators for 25 °C (IMC 18 Heratherm [™]), 50 °C (Thermo Scientific Steri-Cycle CO₂ incubator) samples and a bead bath for 80 °C samples (Lab armour 14L bead bath, M714). Humidity was provided by a saturated salt solution/Cuspor desiccant capsule. A data logger (Hygrochron DS1923-F5) is placed on top of the capsule, which allows for temperature and humidity tracking. Three samples were

placed in each vial: approximately 0.25 g of Small, Medium, and Large crystals (for CBZ dihydrate samples see **Section 2.3.1.3** and **Section 2.3.1.7** for THEO monohydrate samples). Vials were sealed and kept airtight in between sampling time points.

- At 25 and 50 °C, vials were sampled weekly.
- At 80 °C, vials were sampled daily. This was due to quick transitions being seen in lower temperatures showed.

2.7.3. Sampling of Cuspor vials

To sample each vial, the seal was broken on the outer vial by decrimping the aluminium crimp and removing the rubber stopper. Using a claw grip device, each of the three sample vials were removed. The powder in each vial was gently mixed and a sample was then taken from the middle of the vial. A sample size which allowed for DSC (**Section 2.4.1**) and TGA (**Section 2.4.2**) was sufficient. The sample vials were then placed back, by reversing the process to ensure the same vial was at the bottom, middle and top of the large, outer vial. The rubber stopper and a new aluminium crimp was used. The device was securely sealed and placed back into its incubator/oven to continue the experiments.

Note – The sampling process was done as quickly as possible to minimise the changes to the environment within each outer vial.



Figure 2.4: Cuspor Ageing System

2.8. Drying Studies with the DVS Intrinsic

2.8.1. Sample preparation for DVS experiments

Prior to each analysis, the system was calibrated at the experiment's specified conditions with a 100 mg and then an empty pan was tared. After taring, a small amount of sample was placed across the bottom of the DVS pan. **Figure 2.5** below shows the pan, with the dimensions being: A = 1.9 cm, B = 0.7 cm and C = 2.5 cm. Only the flat surface of the pan was used (**B**) and the cake depth for each sample was between 2-3 mm. Dimensions of the pan and cake depth are detailed in **Section 2.9**, and were noted for future

modelling purposes (**Section 6.4.2**). A small, thin layer of sample was routinely used to ensure the entire sample was being exposed as uniformly as possible.

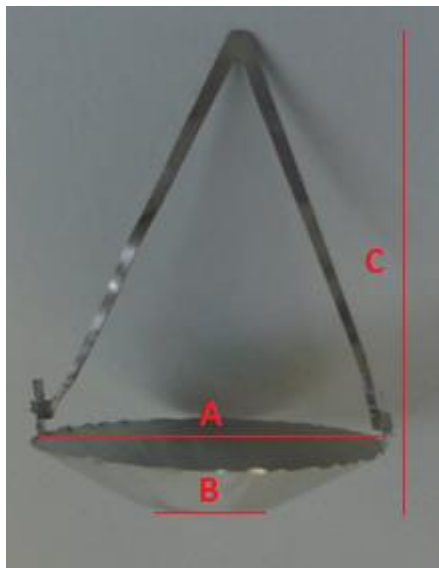


Figure 2.5: DVS Intrinsic sample pan

2.8.2. Drying Studies using the DVS Intrinsic

2.8.2.1. Effects of % Relative Humidity

To investigate the effects of % RH, samples (from **Section 2.3.1.4** and **2.3.1.8**) were analysed at 0, 10, 20, 40, 60 and 80% RH and at 40 °C. The system was calibrated at each set of conditions prior to each experiment. Calibration involved setting conditions (temperature and % RH) and allowing it to stabilise for 30 min. After which, the 'Balance Calibration' wizard in the software was used. The wizard first looks for stability across balance head temperature (set by the system, cannot be adjusted by user), chamber temperature and chamber % RH. Once stability is achieved, a 100 mg weight is added to an empty pan inside the chamber, the door is closed, and the system again looks for stability at the desired conditions. When stability is found again, a mass reading is taken of the 100mg. When you 'Finish' the wizard uses this figure as a

calibration for 100 mg at the desired conditions. Once the wizard is completed and 100 mg removed from the pan, the empty pan is then tared (allowing time for stability at conditions) and the system is ready for a sample. For these studies, the wet sample was held at a specific % RH for 120 min, followed by a 0% RH step. For both model compounds at 40 °C, the 0% RH step was 120 min long. The purpose of the 0% RH step was to ensure the end point sample weight was of the dehydrated or anhydrous form. This was needed for calculating the % moisture content and drying rate of the dry hydrate itself. Samples were analysed in triplicate at each set of conditions.

2.8.2.2. Effects of temperature

To investigate the effects of temperatures, samples (from **Section 2.3.1.4** and **2.3.1.8**) were analysed at 0, 10, 20, 40, 60 and 80% RH at the reduced temperature of 30 °C, for comparison with the same samples analysed at 40 °C. The system was calibrated at each set of conditions prior to each experiment, as detailed above. For these studies, the wet sample was held at a specific % RH for 120 min, followed by a 0% RH step. For CBZ dihydrate, the 0% RH step remained at 120 min, while for THEO monohydrate at 30 °C, this step was increased to 600 min. This increase in time was to ensure the dehydrated or anhydrous form was present at the end of the experiment, resulting in the correct calculations. Samples were analysed in triplicate at each set of conditions.

2.8.2.3. Influence of PSD on drying

Samples of each model compound, with increasing particle size (see **Section 2.3.1.3** for CBZ dihydrate samples and **Section 2.3.1.7** for THEO monohydrate samples) were analysed at 40% RH and 40 °C. The system was calibrated at each set of conditions prior to each

experiment, as detailed previously. These experiments involved holding the wet sample at 40% RH and 40 °C for 120 min, followed by a 120 min 0% RH step. Samples were analysed in triplicate at each set of conditions.

2.8.2.4. Investigating hydrate stability

To investigate hydrate stability, samples of both wet CBZ dihydrate (**Section 2.3.1.4**) and THEO monohydrate (**Section 2.3.1.8**) were analysed at 40 and 60% RH at 40 °C. The system was calibrated at each set of conditions prior to each experiment, as detailed previously. These experiments involved holding the wet sample at a specific % RH for 720 min, followed by a 240 min 0% RH step. Samples were analysed in triplicate at each set of conditions.

2.9. Building the drying model

The first step in building the model was the calculation of the Critical Moisture Content (X_C) and convective heat transfer coefficient (h_C) from the DVS data and other instrument specific values using the Solver function in Excel. The Excel version used was Microsoft® Excel® for Microsoft 365 MSO (Version 2202 Build 16.0.14931.20806) 64-bit. DVS Intrinsic instrument details can be found in **Section 2.4.3**. Instrument specific values used in the model included the sample pan diameter, estimated average cake depth and area of the DVS chamber. The sample pan used in the DVS is shown in **Figure 2.6**. While the pan has a diameter of $A = 1.90$ cm, only the flat surface along the bottom (**Figure 2.6 (2)**) was used during sample preparation for the small-scale drying studies. This flat surface has a diameter of $B = 0.7$ cm, which was the value used for calculations. The length/height of the entire pan was $C = 2.5$ cm but for both hydrate forms, the cake depths in experiments ranged from 0.20 - 0.30 cm so a value 0.25 cm was taken for

calculations. The area of the DVS chamber was 40 mm x 40 mm x 50 mm (W x D x H) and its set gas flow of 200 sccm (0.00188 kgm^{-3}).

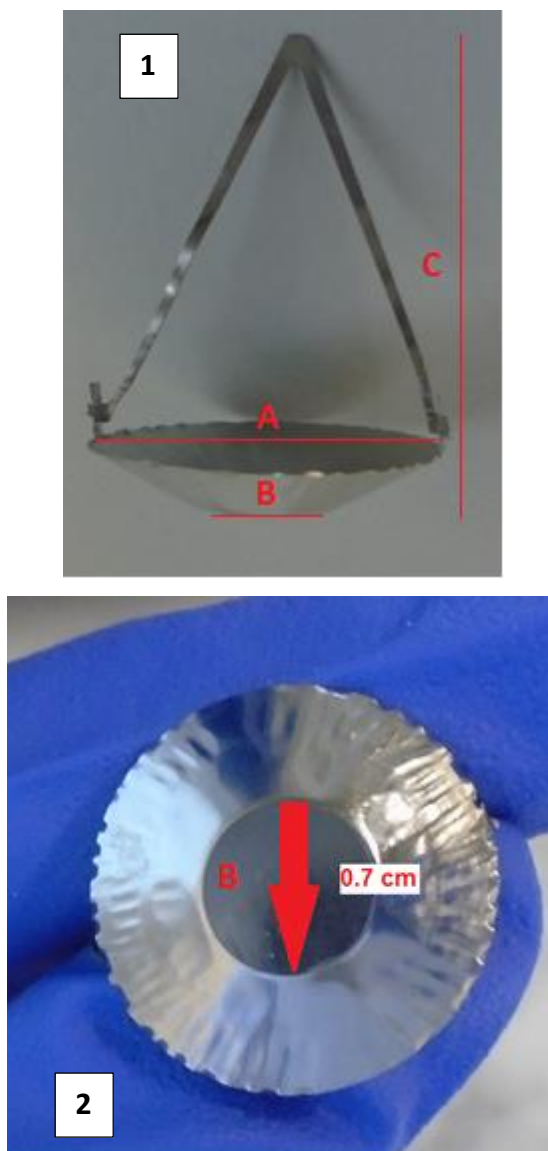


Figure 2.6: Image of the DVS Intrinsic sample pan, showing dimensions. **(1)** showing the length/height perspective and **(2)** showing the underside of the pan.

For the **oven** drying time prediction aspect of the model, there are several user inputs that are required:

1. Initial Moisture Content (%)
2. Measured Relative Humidity set outside the oven (% RH)

3. Oven pressure (bar)
4. Initial sample mass (g)
5. Gas temperature (°C)
6. Internal % RH – separately calculated

A Binder VDL 23 (version: VDL023-230V) was the vacuum oven used in for this work. Initial Moisture Content of the sample was determined using the Moisture Analyzer, as per **Section 2.5.2**. % RH, oven pressure and temperature are values which are set outside the oven, see **Section 2.10.1.2** for more details. The diameter of the weigh boat used had a value of 5.00 cm. The initially determined X_c and h_c values (from DVS data) are used to predict the drying time in the oven. While X_c remains the same (i.e. the value used to model oven drying is the same as that calculated from DVS data), the h_c must be scaled to match the oven conditions as it is a factor of the gas flow rate in the oven. The scale of the gas flowrate in the oven is compared to the DVS. Oven specific details include its dimensions; 285 mm x 295 mm x 285 mm (W x D x H). Other oven system setup details like wet and dry line velocities are detailed in **Section 2.10.1.2**.

2.10. Scaled-up oven drying studies

2.10.1.1. Experimental Setup

Figure 2.7 shows the piping and instrumentation diagram (P&ID) for the scaled-up oven drying setup. House N₂ is used and is split into a Dry nitrogen (N₂) line and Wet N₂ line. The dry line immediately feeds into a flow meter (Key Instruments MR3000 Series Variable Area Flow Meter for gas, 0.4 litres per minute (LPM) minimum, 5 LPM maximum). The wet line is first fed into a Dreschel gas washing bottle. The output line then feeds into a separate, but identical flow meter as the dry line. The two streams of nitrogen

come together using a union piece and feed into one port of a three neck round bottom flask. The flask contains a Vaisala Humidity and Temperature Transmitter (HMT333) to determine the % RH levels of the N₂ mixture prior to entering the oven. Finally, the output line on this flask feeds directly into the oven. The oven is a Binder VDL 23 which is connected to an Edwards RV3 vacuum pump.

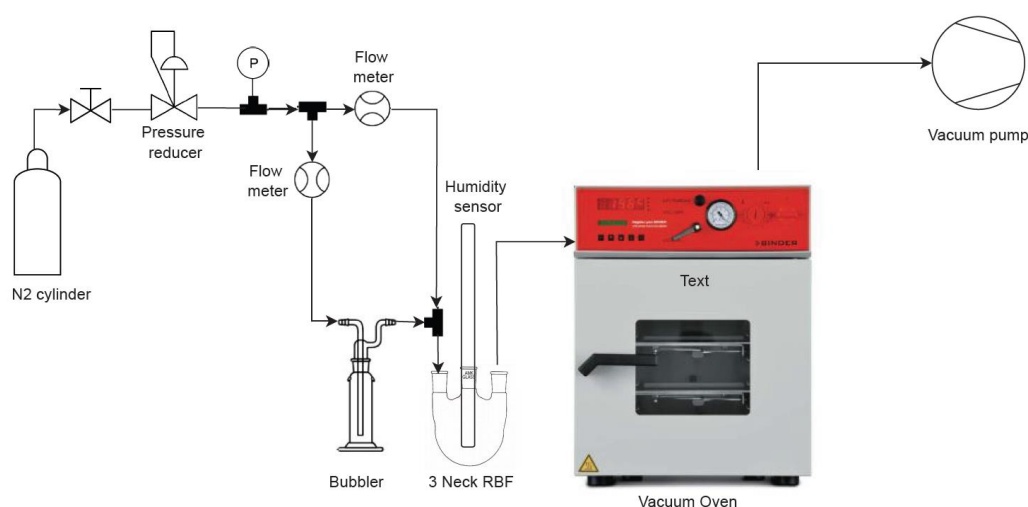


Figure 2.7: P & ID of the scaled -up drying setup.

2.10.1.2. System setup

When setting the temperature of the oven, an additional 3 °C is added to overcome heat transfer inside the oven to the sample. Differences in temperature were noted between what was set on the interface of the oven and what was recorded using a thermometer inside the oven. By increasing the temperature 3 °C higher than the desired temperature, the correct temperature was consistently seen inside. Where changes in temperature were needed before an experiment, the system was given a minimum of 1 hr to stabilise after reaching the desired temperature.

For % RH levels, both flow meters were adjusted with the ratios of dry to wet determining the level of RH. For example, if both lines

were at 1 LPM, this consistently gave the correct reading of 50% RH. A total gas flow rate of 2.0-2.5 LPM was used throughout all experiments. % RH levels were allowed to stabilise for a minimum of 1 hr before conducting experiments.

2.10.1.3. Sample preparation for scaled-up oven drying experiments

Batches of 'wet' hydrate cakes were generated as per **Section 2.3.1.4** and **2.3.1.8**. Sample preparation firstly involved collecting % Moisture Content data for the bulk cake using the Moisture Analyzer - see **Section 2.5.2** for details. Then 0.5-1.0 g of wet cake was spread out into a weigh boat, ensuring to only cover the flat bottom. Large, agglomerated particles were gently broken apart to form a thin, single layer of sample across the bottom of the weigh boat. Sample weight was confirmed using a Mettler Toledo MS105 balance. To align this work, with that of the drying studies in the DVS, efforts were made to ensure this layer was as thin as possible to reduce the impact of diffusion of moisture through the cake on drying. Estimations on the cake depth and the diameters measurement of the weigh boat were noted for modelling purposes. The weigh boat was then covered with a piece of tissue and held in position with adhesive tape. Having the tissue in place prevents sample loss when the vacuum is released at the end of each experiment. Pre-drying % M.C. and initial sample weight are required for the predictive model.

2.10.1.3.1. Sample and system preparation for model input details

The model requires the below inputs:

- Temperature (°C)

- % RH (Antoine equation used to calculate % RH inside the oven based on conditions set outside; % RH, temperature, and pressure)
- Sample weight
- % Total Moisture Content prior to drying

Temperature and % RH were captured as detailed in **Section 2.10.1.2**. A sample of the bulk sample cake was analysed by the Moisture Analyzer (**Section 2.5.2**) to get its % total Moisture Content prior to drying. Sample weight was then obtained during its preparation as detailed in **Section 2.10.1.3**, and was also taken at the end of the experiment to compare theoretical and actual weights of the dry hydrated forms. All values are inputted into the model (**Section 2.9**) to give a predicted drying time.

2.10.2. End point sample analysis

At the end of the experiment, the sample was removed and weighed on a Mettler Toledo MS105 to confirm a final sample weight. This can be compared with the theoretical dry weight of the hydrate form calculated by the model. % M.C. was obtained using the Moisture Analyzer - see **Section 2.5.2** for details. XRPD analysis was then used to confirm the form of the end point sample, as per **Section 2.4.5**.

CHAPTER 3. Crystallisation processes and other methods used for the robust generation of polymorphs, hydrate forms, and hydrate crystals of varying particle size for both model compounds

3.1. Abstract

This chapter details the generation and characterisation of the hydrate forms of both CBZ and THEO. The crystallisation processes were monitored by PAT, using PVM technology to track the conversions. The production and characterisation of other polymorphs of each compound are also detailed. The aim of this work was to build a library of results which could be used as a reference point for form determination throughout the research detailed in this thesis.

Both hydrates were successfully generated, CBZ dihydrate by slurry conversion and THEO monohydrate by cooling crystallisation. Water was the only solvent used in the crystallisation work. Tracking of the CBZ conversion with PVM showed the clear change in crystal habit to the hydrated form and indicated that the conversion to the hydrate form took less than the approximate 24 hr reported in the literature [20, 38, 98]. The THEO conversion showed only subtle morphological changes, with generation of the monohydrate being confirmed through off-line solid-state characterisation. Polymorph generation was confirmed through the comparison of solid-state analysis results with those detailed in the literature.

Finally, several methods were investigated for the generation of hydrate crystals of varying size. Solubility data was gathered for both compounds; CBZ dihydrate data was obtained using the gravimetric method, while the Crystal16™ equipment setup was

used for THEO monohydrate. CBZ dihydrate proved to have very poor solubility in water, hence the use of the gravimetric method. Although poorly soluble, the use of a thermocycling method successfully produced CBZ dihydrate with larger PSDs when compared to crystals isolated via the standard method. To generate “small” CBZ dihydrate crystals, the mechanical method of milling was used on a previously generated batch of dihydrate crystals, which resulted in a reduction in PSD values. In the case of THEO, the initial cooling crystallisation method for THEO monohydrate was altered, using different cooling rates, to successfully produce crystals of smaller and larger size.

3.2. Introduction

CBZ and THEO each have four polymorphs (I, II, III and IV) and a dihydrate and a monohydrate form, respectively. Both model compounds, when in their hydrated forms are known channel hydrates.^[31, 75, 84, 86] The generation of both hydrate forms was integral to this project and establishing robust crystallisation procedures was an appropriate starting point.

As discussed in **Section 1.1.2.1**, the pharmaceutical industry often considers the incorporation of water into the crystal lattice to be more acceptable than other solvents, as it is unlikely to increase toxicity levels associated with an active pharmaceutical ingredient (API) when compared to other solvents. Water offers unique chemistry when incorporated into the crystal lattice, as it consists of two positive and two negative charge regions ^[14, 99], meaning strong interactions and hydrogen bonding with API(s) are common. A singular water molecule can be responsible for a coordinate covalent bond, hydrogen bonding or van der Waals interactions with API(s) and excipient(s) ^[99].

Hydration in hydrates tends to occur via solution or solution-mediated mechanisms and involves water being associated as part of the crystal packing. In channel hydrates, this hydration occurs because the water molecules forms direct intermolecular hydrogen bonds to create channels or tunnels ^[100]. These bonds are stronger than that of water-water bonds and physiochemical properties of the API can be affected e.g. higher melting and boiling points, changes in density and absorbance spectra. Channel hydrates are also known to be affected by temperature and humidity ^[101], leading to variability in composition of solids, and is a reason why channel hydrates are often avoided as the selected API form for a drug product.

CBZ dihydrate can be formed in aqueous solutions via a solution-mediated mechanism. Depending on the starting polymorph, the kinetics of the transformation can vary. The resulting hydrate has a monoclinic structure. The structure consists of a dimer motif between carboxamide groups, a proton donor (NH₂) and a proton acceptor (C=O). ^[102] A network of hydrogen bonding connects the remaining sites of the amido group and the water molecules, linking the dimers into a double layer. There are 4 parallel molecules of water in each layer of the dihydrate lattice, which form channels along the C-axis.

The generation of THEO monohydrate results in a monoclinic form. THEO molecules are surrounded along the *a* axis with water molecules, forming infinite chains by hydrogen bonding. The chains are also crosslinked through hydrogen bonds by hydrogen-bonded theophylline dimers. ^[87] The monohydrate packing consists of hydrogen bonded theophylline dimer pairs that are connected through additional hydrogen bonding with water molecules to form infinite chains.

A slurry conversion was previously used to generate CBZ dihydrate from the commercial anhydrous CBZ [20, 38, 98] and involved a 24 hr hold time. Slurry conversions are commonly used during polymorph screening. It is a dynamic process, whereby the more soluble form partially dissolves and the less soluble form precipitates [103].

The conversion of anhydrous THEO to its monohydrate was previously reported, and involved a cooling crystallisation [86]. Solubility increases with temperature. At a specific temperature, the solution becomes saturated, and the maximum amount of solute has been dissolved in the solvent. When saturated mixtures start to cool, they become supersaturated, and crystallisation then begins. As the jacket temperature of the crystalliser reduces, crystals begin to form with the stir shaft continuously re-introducing them into the slurry, encouraging crystal growth. Cooling crystallisations are known to have high uniformity of crystal size which can be manipulated. By changing the cooling rates, the PSD of crystals can be impacted. The faster the cooling rate, the smaller the crystals that will be generated.

Solid-state characterisation methods, including, but not limited to, DSC, DVS, microscopy, TGA, SEM and XRPD, were established and used to characterise the various solid-state forms of the two APIs generated in the current work.

3.3. Results and Discussion

3.3.1. Hydrate formation

3.3.1.1. CBZ Dihydrate

The CBZ dihydrate form was prepared using a slurry conversion method, employing a 24 hr hold time, as described in **Section 2.3.1.2**. SEM and optical microscopy (**Figure 3.1**) showed the crystal habit had changed from small (<10 μm) irregular crystals for the anhydrous CBZ starting material, to needle-like crystals, representative of the dihydrate.^[104] The successful conversion was confirmed with XRPD (**Figure 3.2**). The generated CBZ dihydrate showed a pattern which differed to the anhydrous CBZ. The dihydrate trace compared well with those reported in the literature, including matching characteristic peaks^[75].

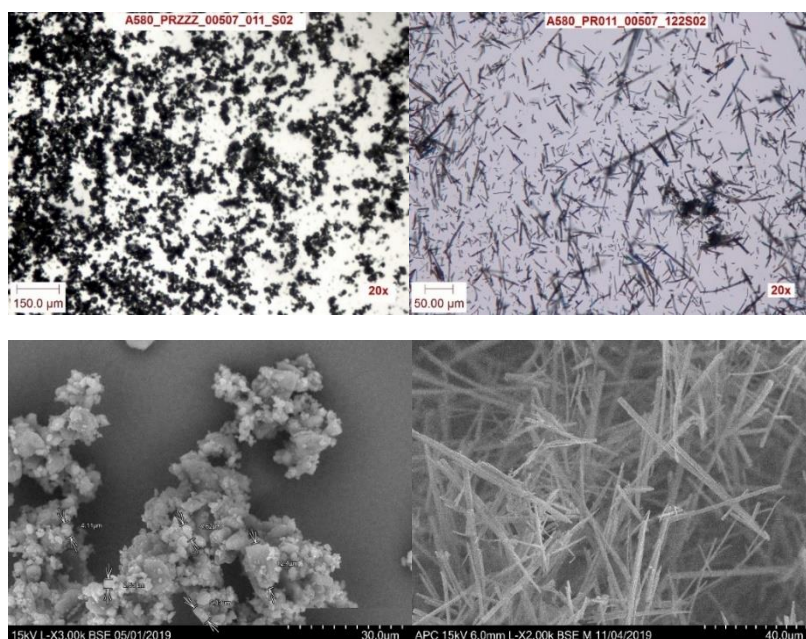


Figure 3.1: Light microscopy images (top) and SEM images (bottom) of anhydrous CBZ, Form III (left) and CBZ dihydrate (right).

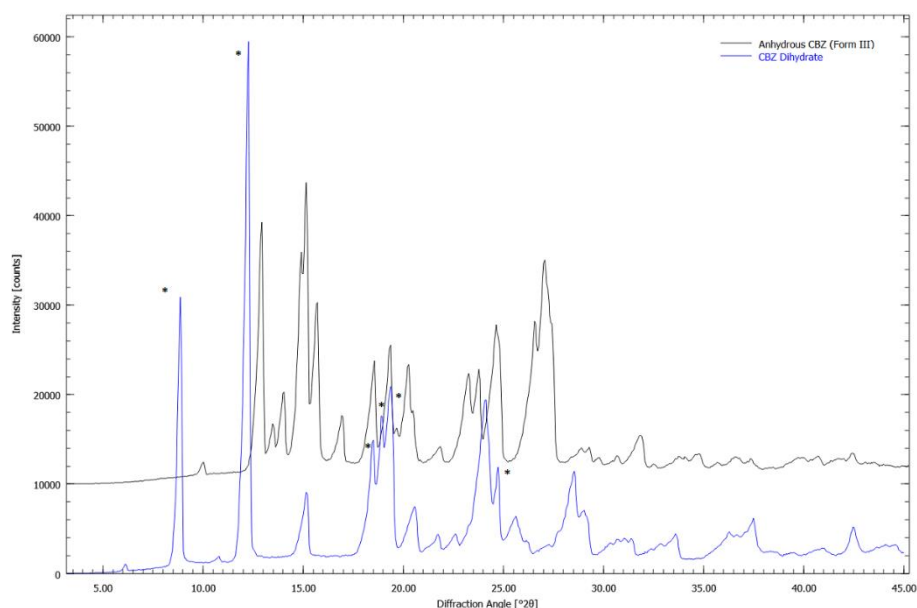


Figure 3.2: XRPD of anhydrous CBZ, Form III (black) and CBZ dihydrate (blue). * denotes the characteristic peaks of the dihydrate form.

DVS isotherms are shown in **Figure 3.3**, and demonstrate clear differences in the sorption/desorption behaviours of the anhydrous CBZ and the dihydrate form. The large hysteresis, the gap between the sorption and desorption plots, are characteristic of hydrate formation. ^[105] Sorption values acquired from DVS analysis can provide an insight into whether the moisture present is likely to be adsorbed onto the surface or absorbed within the bulk of the solid and may be used to determine hydrate stoichiometry. DVS can also be used in conjunction with DSC and TGA to provide information on dehydration, polymorphic transitions, and stability control.

Anhydrous CBZ was analysed via DVS in duplicate and the sample shown in **Figure 3.3** (left) had a sorption value of $0.038\% \pm 0.021$. The generated dihydrate was also analysed in duplicate and results in **Figure 3.3** (right) showed a value of $13.46\% \pm 0.73$. Based on the stoichiometric values, the theoretical value of 13.22% would indicate the presence of the dihydrate form. Based on the results below, the dihydrate presence was confirmed. **Figure 3.4** shows a

sample of CBZ dihydrate (from the same batch as previously discussed) going through two sorption and desorption cycles. The crystal lattice sorbs and desorbs the correct amount of water during the first cycle, however during the second cycle, no water sorption takes place showing that the crystal lattice has collapsed and that it cannot remain stable under numerous cycles.

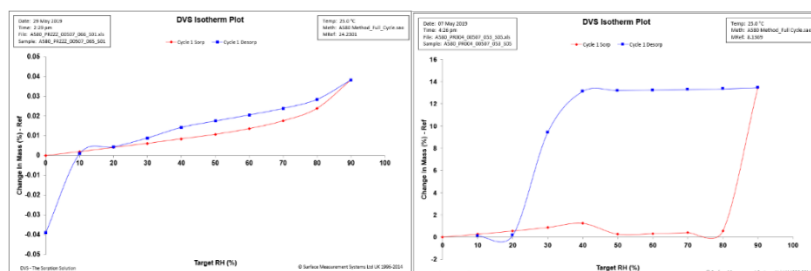


Figure 3.3: DVS isotherm plots of anhydrous CBZ, Form III (left) and CBZ dihydrate (right).

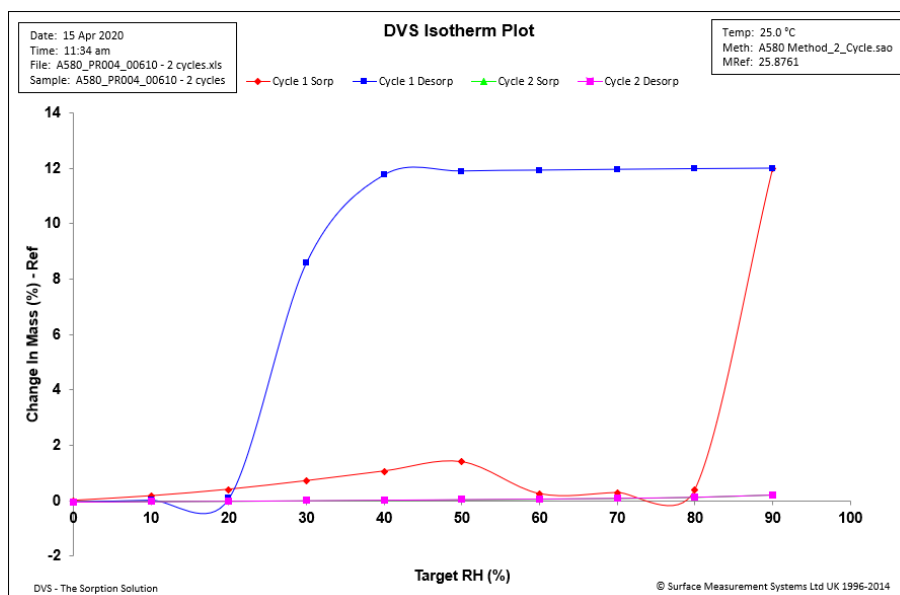


Figure 3.4: DVS isotherm plot of CBZ dihydrate going through two sorption and desorption cycles.

Figure 3.5 shows the DSC thermograph overlay of anhydrous CBZ and the dihydrate. For the anhydrous starting material, DSC analysis was carried out in triplicate. Each graph overlaid well and compared well with the literature. [78, 104] Each thermogram contained two endotherms, the first being related to the Form III

melt and the second being the characteristic Form I melt. The literature describes a small exotherm occurring between the endotherms^[78], which denotes the crystallisation of Form I prior to its melt. However, in each of the triplicate analyses, this exotherm was not obvious. **Figure 3.6** shows a zoomed in section of this thermogram, attempting to show the exotherm. Despite the evidence being difficult to see, as the characteristic melt for the Form I polymorph was present, it proved the crystallisation did occur. For CBZ dihydrate, its thermogram shows an endotherm with an onset of 70 °C which is the peak of hydration for the hydrate form. This then converted, on heating, to Form I, with its characteristic melt being seen at ~190 °C.

TGA analysis of the anhydrous starting material was carried out in triplicate with each graph being the same as depicted in **Figure 3.7** (left), with no feature other than degradation. For the dihydrate, analysis was carried out in duplicate with both analyses showing a step-like feature with % weight loss values close to the theoretical 13.22% value of the dihydrate form. The sample shown in **Figure 3.7** (right) had a value of 13.03% $\pm$ 0.07, further confirming the presence of the dihydrate form.

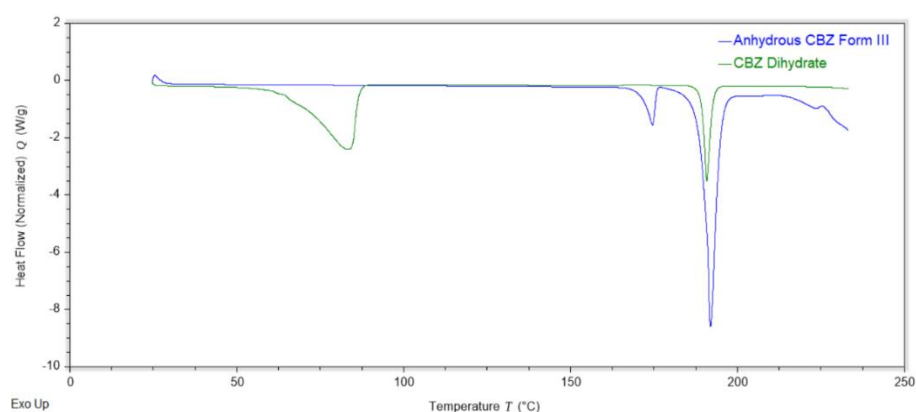


Figure 3.5: DSC overlay of anhydrous CBZ, Form (blue) and CBZ dihydrate (green).

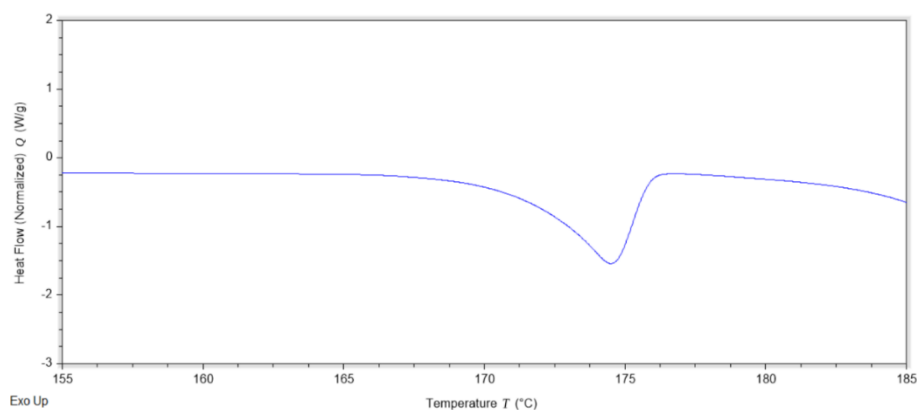


Figure 3.6: A zoomed in section of the anhydrous CBZ DSC thermograph, showing the small exotherm of the Form I crystallisation.

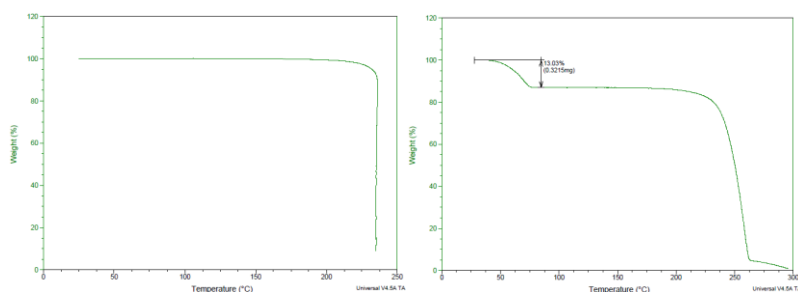


Figure 3.7: TGA analysis of anhydrous CBZ, Form III (left) and CBZ dihydrate (right).

The slurry method used for generating the dihydrate has been well documented [20, 38, 98] with reference to the 24-hr agitated hold. However, one previous publication [106] detailed how this conversion from the anhydrate to dihydrate was at 95% completion after the first hour.

While undertaking a preliminary experiment designed to analyse the effects of thermocycling (**Section 3.3.4.2**), this conversion to the hydrate from was monitored in more detail. With the new model PVM which was utilised (**Section 2.6.2**), auto focusing during an experiment was possible. This allowed for detailed images to be captured throughout the experiment, as shown in **Figure 3.8**. Older PVM models (**Section 2.6.2**), when used, were unable to capture the transition from small, irregularly shaped particles to the needle

habit of the dihydrate due to the dihydrate form being out of focus once the transition began. PVM analysis and the captured images (**Figure 3.8**) showed that the conversion of CBZ Form III to CBZ dihydrate was rapid and much of the conversion had taken place within 25 min. The large, white unfocused shapes seen in the background of the images in **Figure 3.8** are large agglomerates. Taking a closer look at the 'start' image, on the top left of **Figure 3.8**, the needle-like structure, representative of the dihydrate, could be measured and was found to be $\sim 1\ \mu\text{m}$ thick, demonstrating the resolution of this new piece of equipment. By incorporating this new PVM technology into these experiments, it was found that needle like crystals of the dihydrate were seen instantly and the conversion neared completion at approximately 25 min.

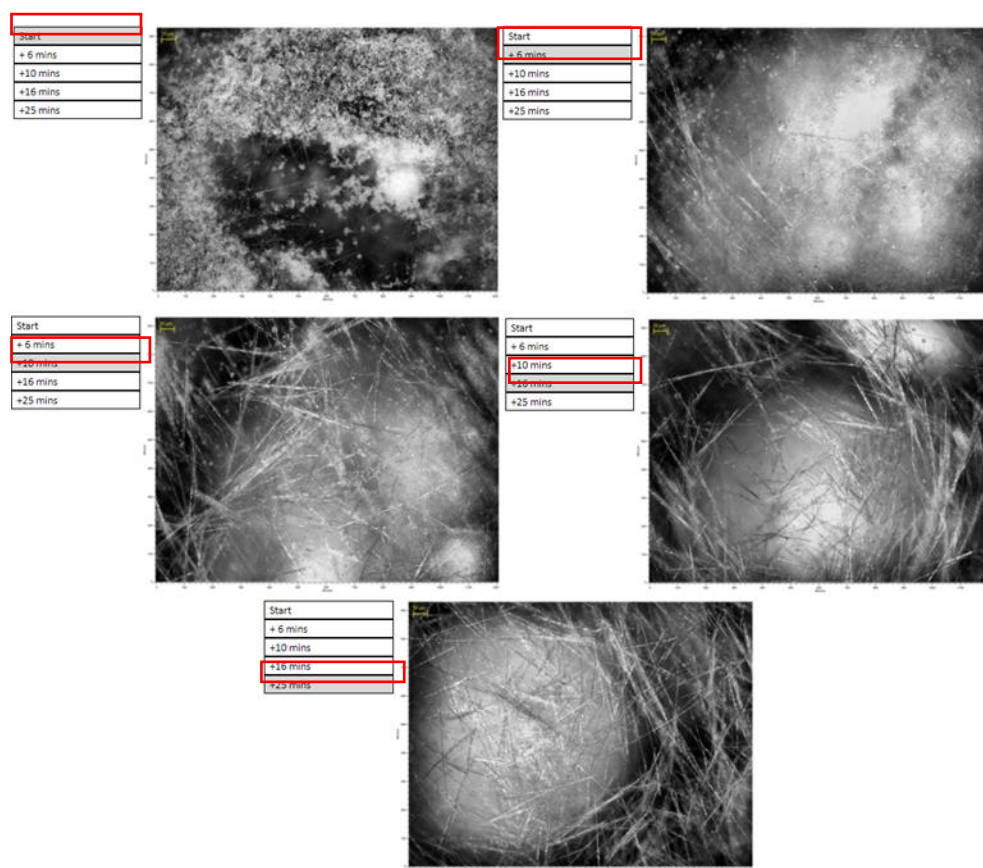


Figure 3.8: PVM, EasyViewer 400: 25 min time lapse of the anhydrous CBZ, Form III conversion to CBZ dihydrate.

The change in aspect ratio during this initial conversion time was plotted (**Figure 3.9**). The aspect ratio, which is the length divided by the width, gives an indication of the particle shape. By plotting this parameter over time, it showed a shift in the curve towards the left meaning that, over time, the particles become longer and narrower. Using the time stamps on the legend in **Figure 3.9**, we see that the shift to the left increases with each time point but begins to stabilise (shown by the minimal changes in the curve position) between 16 min and 25 min.

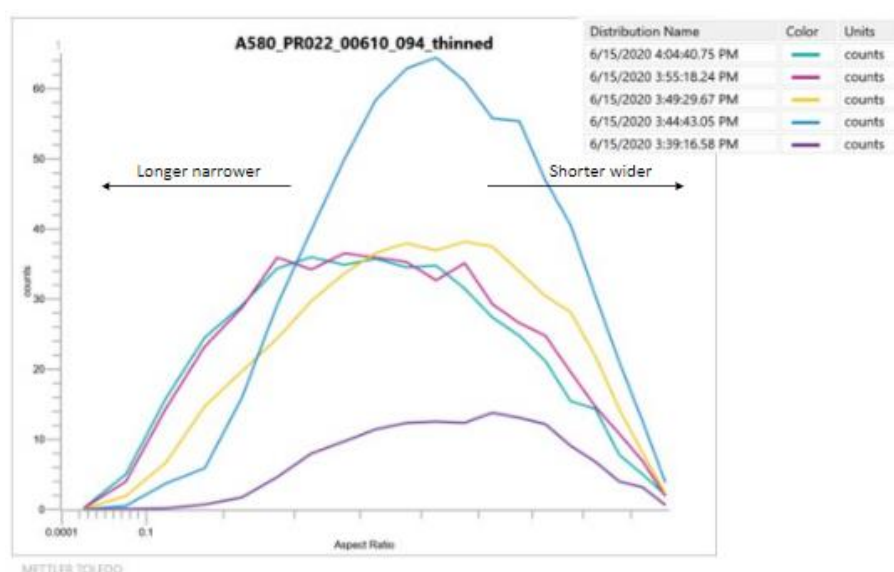


Figure 3.9: Changes in aspect ratio during the initial conversion of the anhydrous CBZ, Form III to CBZ dihydrate.

3.3.1.2. THEO Monohydrate

In the case of THEO, the difference in crystal morphology between the anhydrous and hydrate forms was more difficult to differentiate by microscopy, compared to those of CBZ. SEM and optical microscopy showed that crystals changed from initial Form II thicker plate-like crystals^[86] to thinner, needle-like crystals, of the hydrate^[107] (**Figure 3.10**).

The conversion to THEO monohydrate was confirmed with XRPD (**Figure 3.11**). A clear distinction between the anhydrous solid and the hydrate was evident with the characteristic peaks detailed in the literature ^[108] being present in the hydrate sample.

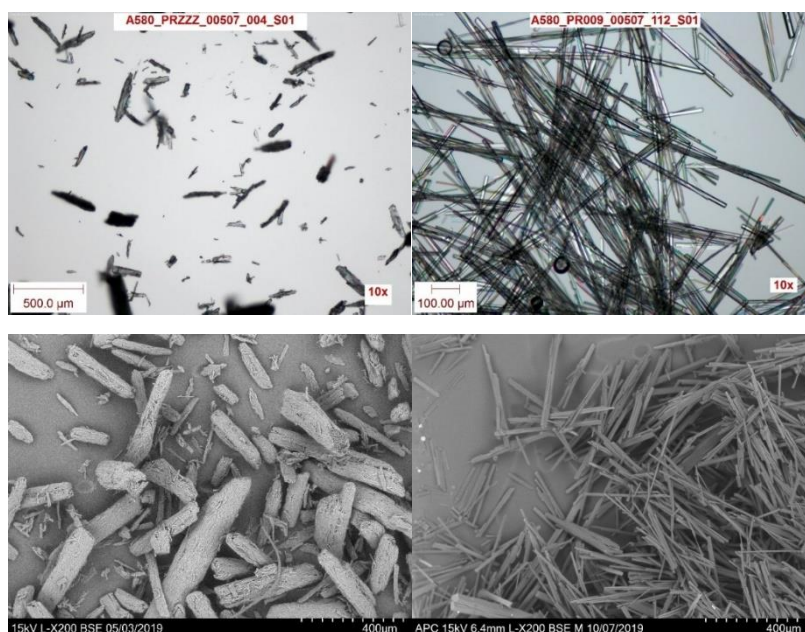


Figure 3.10: Light microscopy (top) and SEM (bottom) images of anhydrous THEO, Form II (left) and THEO monohydrate (right).

DVS analysis (**Figure 3.12**) also allowed for the stoichiometry of the hydrate to be confirmed. Anhydrous THEO (Form II) had a maximum sorption value of 0.054 %, which was an expected result for an anhydrous, non-hygroscopic solid. The generated hydrate had a sorption value of 9.13 % which equates closely to the theoretical value of 9.08% associated with the sorption of one molecule of water per molecule of API. The “bump” at 40 % RH on the hydrate DVS isotherm is characteristic of THEO monohydrate, as detailed in an application note from the instrument manufacturer ^[109]. It is an interesting characteristic which has been investigated in literature ^[110], and was determined to relate to a lattice transition from the dehydrated form to the anhydrate form, whereby the absorption of water between 30-50% RH resulted in a

crystallisation process. Whether this was due to water being incorporated into amorphous regions or into spaces left by desorbed water in a 'ghost hydrate' structure is still unclear. **Figure 3.13** shows a sample of THEO monohydrate (from the same batch as previously discussed) going through two sorption and desorption cycles. The crystal lattice sorbs and desorbs the correct amount of water in both cycles, showing the crystal lattice was stable when water is removed and re-introduced several times.

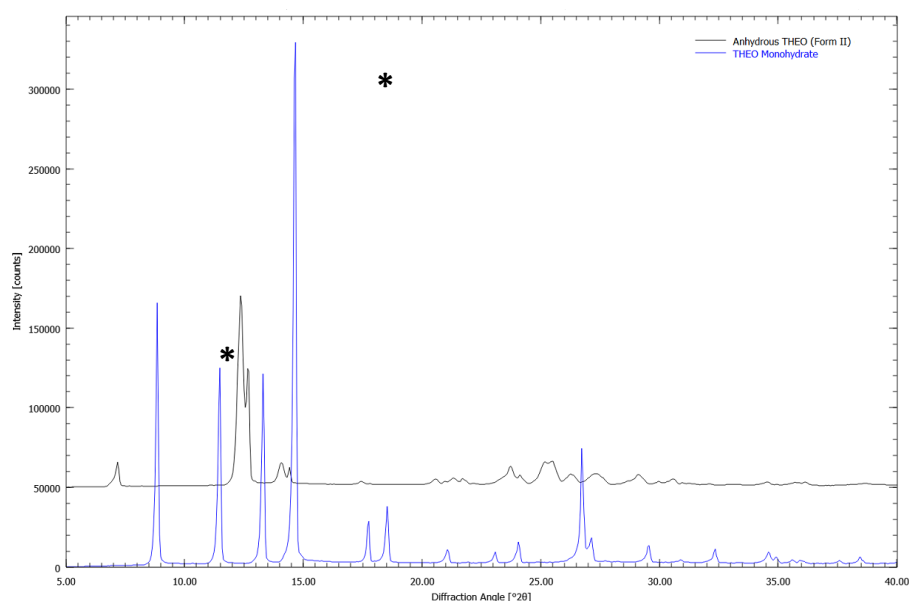


Figure 3.11: XRPD of anhydrous THEO, Form II (black) and THEO monohydrate (blue). * denotes the characteristic peaks of the monohydrate.

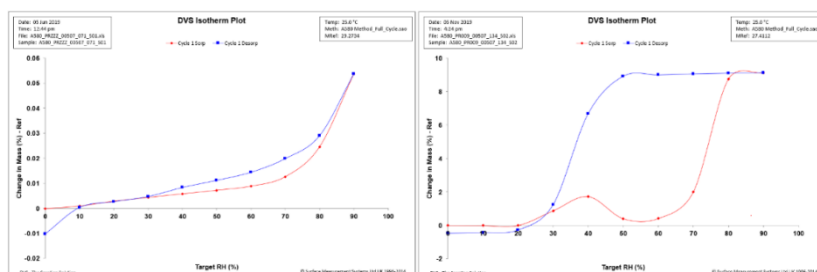


Figure 3.12: DVS isotherm plots of commercial THEO Form II raw material (left) and generated THEO monohydrate (right).

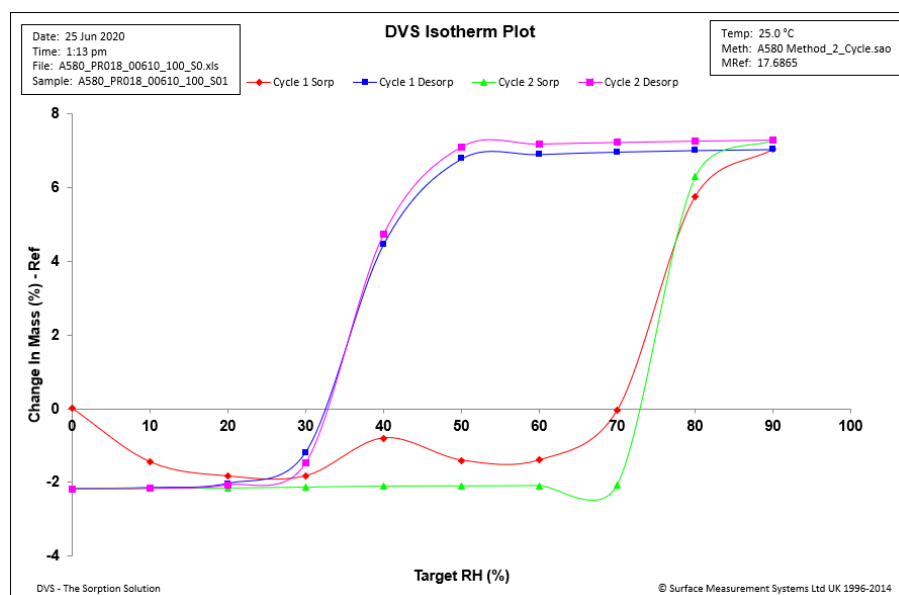


Figure 3.13: DVS isotherm plot of THEO monohydrate going through two sorption and desorption cycles.

DSC thermograms (**Figure 3.14**) showed the expected peak of hydration for the monohydrate sample, followed by the characteristic Form II melt at ~ 272 °C. TGA results (**Figure 3.15**), showed only degradation for the anhydrous sample and this was seen in each of the triplicate results for the starting material. For the monohydrate sample, a clear step was observed with a weight loss of $\sim 9.08\% \pm 0.07$. This weight loss equates to the loss of one water molecule, agreeing with the previously discussed DVS data. Analysis of the generated monohydrate was carried out in duplicate.

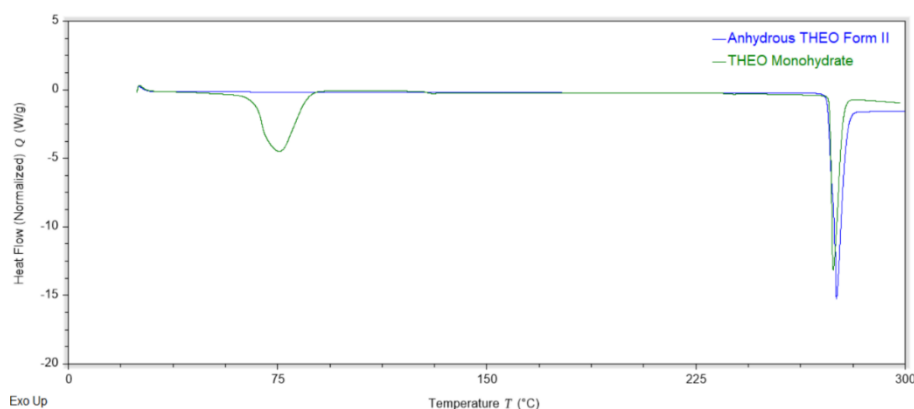


Figure 3.14: DSC overlay of anhydrous THEO, Form II and THEO monohydrate.

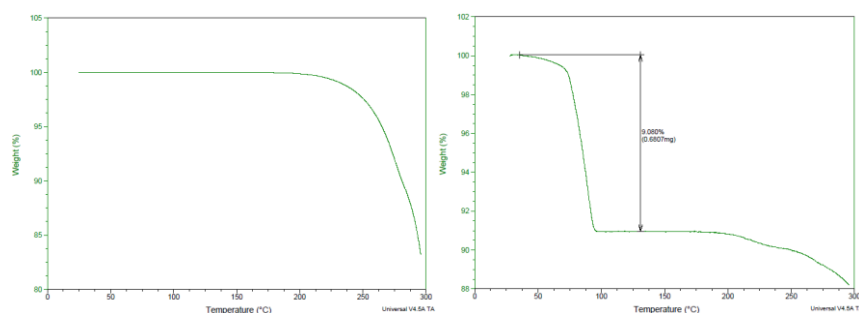


Figure 3.15: TGA traces of anhydrous THEO, Form II (left) and THEO monohydrate (right).

3.3.2. CBZ Polymorphs

The generation of CBZ polymorphs are detailed in **Section 2.3.1.5**. Solid-state analysis of each generated form was performed to build a library of results, which could be used for future form identification.

3.3.2.1. Anhydrous Form I

A method from the literature was used ^[76] and optimised for the generation of the anhydrous Form I polymorph. Agitation proved to be an important parameter in the conversion from the anhydrous Form III starting material. Optimisation was required to ensure full conversion as initial attempts resulted in mixtures of polymorphic forms being produced, and insufficient mixing was

determined to be the likely issue. By using an EM system, it allowed for more efficient mixing and better heat transfer. Agitation was set to 200 rpm and T_r (reaction temperature) was set to 140 °C for 5 hr (**Section 2.3.1.5.1**). By setting the T_r temperature, this ensured that the jacketed temperature was high enough to bring the solid in the vessel to the required 140 °C. Efficient heat transfer resulted in consistent conversion to Form I.

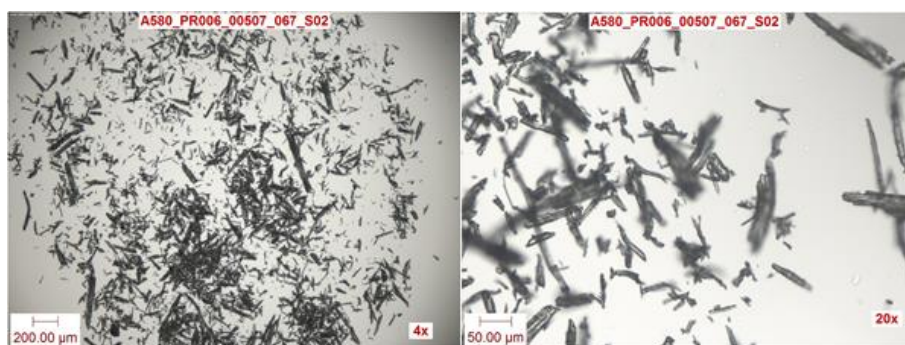


Figure 3.16: Light microscopy images of anhydrous CBZ, Form I.

Form I crystals have a plate-like morphology which differs from the small irregular crystals of the commercial anhydrous Form III (**Figure 3.1** (left)). Microscopy images (**Figure 3.16**) showed no evidence of the original Form III crystals, demonstrating a successful conversion to a new form. The XRPD patterns of Form I (**Figure 3.17**) differs greatly to the original Form III starting material and also compares well with Form I patterns reported in the literature. ^[78] To further confirm the presence of Form I, DSC analysis was undertaken. The DSC thermogram (**Figure 3.18**) was representative of Form I, showing only a single endotherm at approximately ~190 °C. The melt temperature and lack of other features confirms the presence of only the Form I polymorph. TGA analysis showed only degradation (**Figure 3.19**), which is to be expected for an anhydrous form.

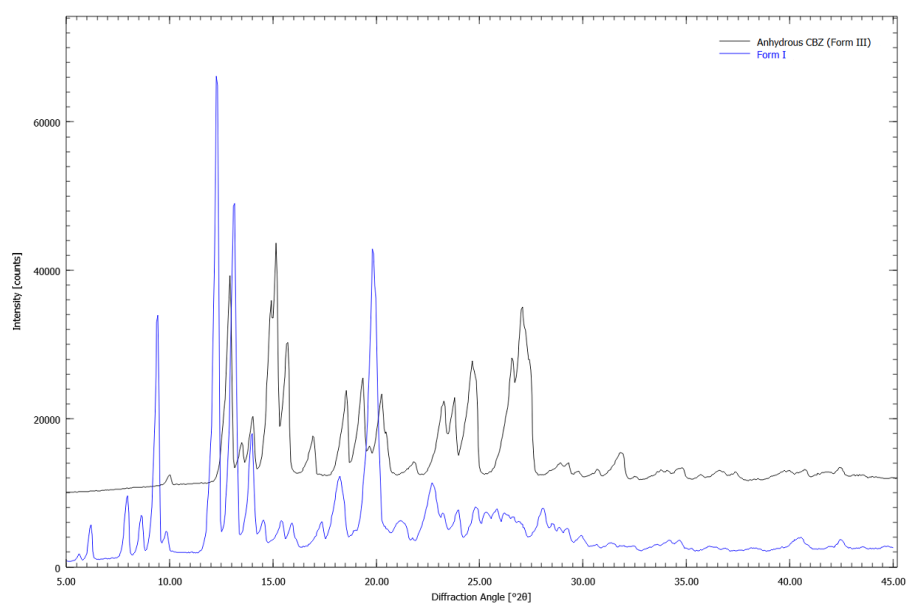


Figure 3.17: XRPD overlay of anhydrous CBZ, Form III (black) and anhydrous CBZ, Form I.

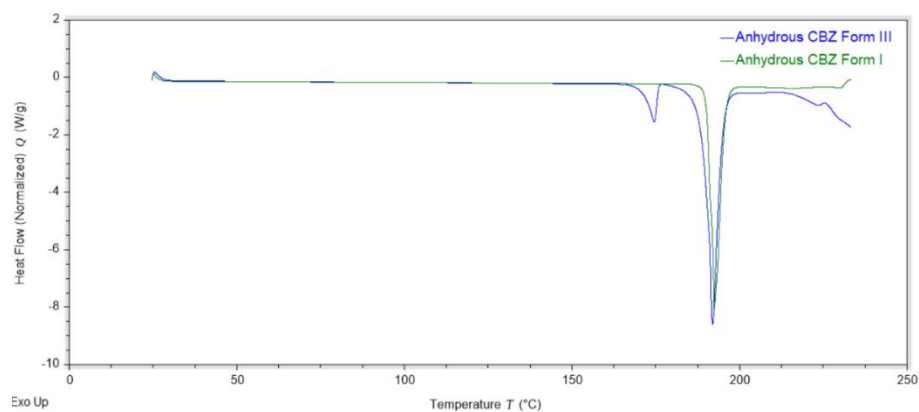


Figure 3.18: DSC thermogram overlay of anhydrous CBZ, starting material and CBZ, Form I.

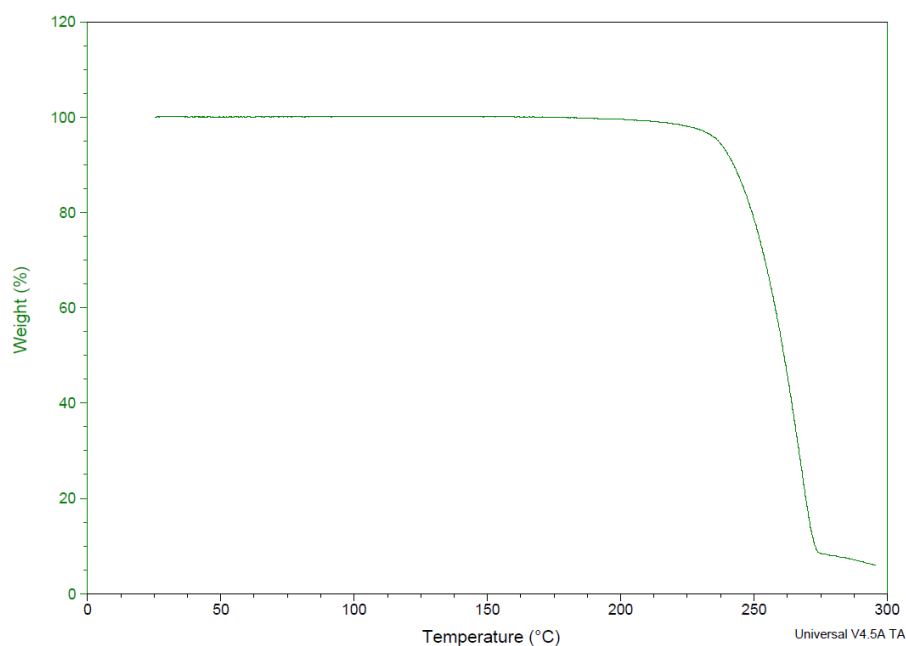


Figure 3.19: TGA graph of anhydrous CBZ Form II.

3.3.2.2. Anhydrous Form II

Production of this polymorph was undertaken by means of a slow cooling crystallisation with ethanol, based on a method from the literature,^[78] with additional details provided in **Section 2.3.1.5.2**.

Microscope images of the resulting solid (**Figure 3.20**), showed clear differences to the Form III starting material (**Figure 3.1** (left)). While crystal size remained small, the morphological change was clear, with most crystals transforming to needle-like structures. Small amounts of the original, irregular crystals of Form III were observed, suggesting that a mixture of forms was present and full conversion did not take place.

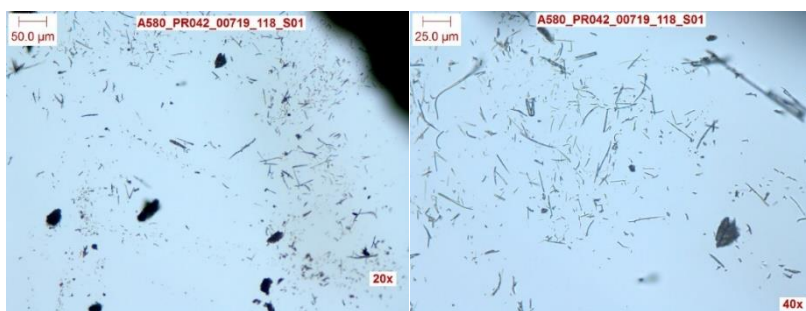


Figure 3.20: Light microscopy images of the sample crystallised in an attempt to prepare anhydrous CBZ, Form II.

XRPD analysis of this sample was completed using the new XRPD available (**Section 2.4.5**). The XRPD pattern of the sample overlayed with the Form III starting material (**Figure 3.21**) while also showing some new peaks. Some Form II peaks listed in the literature [78] were found at 13.21, 18.44, 20.07, 21.13, 22.93, 23.35, 25.46, 28.61, 29.37 and 32.60° 2 θ . Numerous attempts to generate the pure Form II anhydrous material consistently resulted in a sample containing a mixture of forms.

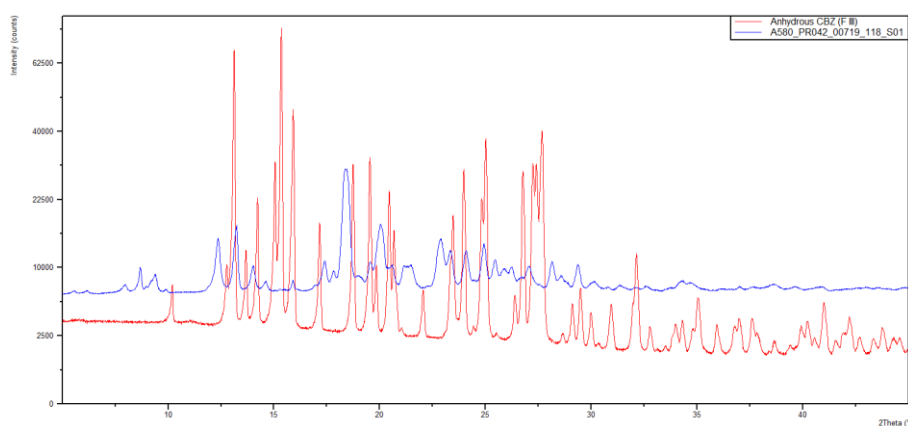


Figure 3.21: XRPD overlay of anhydrous CBZ, Form III (red) and the sample crystallised in an attempt to prepare anhydrous CBZ, Form II (blue).

DSC analysis was then completed, with the overlay of the sample generated and the Form III starting material shown in **Figure 3.22**. While Form II does not melt within the temperature range of 135-170 °C, the sample generated shows a subtle disturbance to the

baseline, which is associated with a type of transformation which has been shown in literature. [78] The melt of this new phase then occurs between 188 and 192 °C.

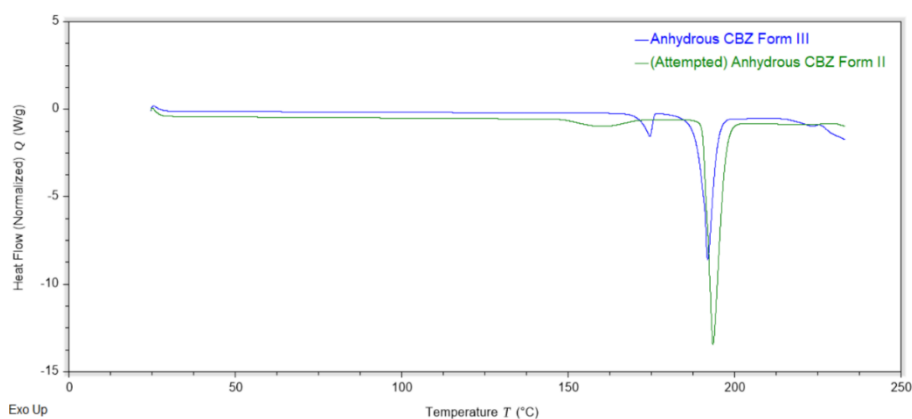


Figure 3.22: DSC thermogram overlay of anhydrous CBZ, Form III (blue) and the sample crystallised in an attempt to prepare anhydrous CBZ, Form II (green).

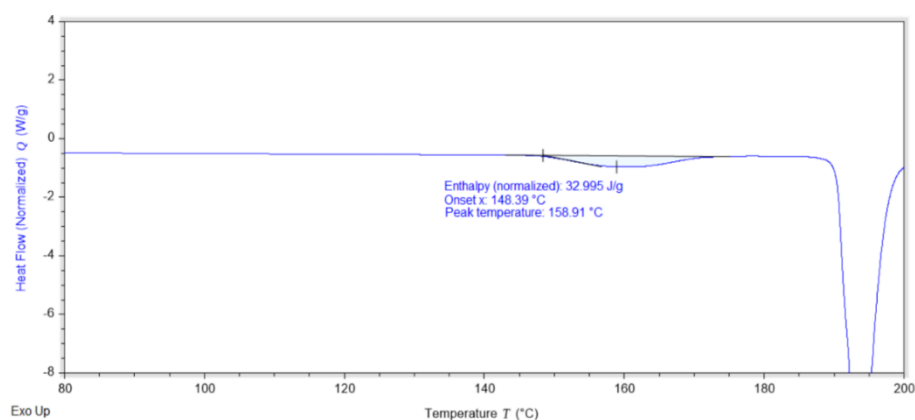


Figure 3.23: Zoomed in section of the DSC thermogram for the sample crystallised in an attempt to prepare anhydrous CBZ, Form II (green).

TGA analysis (**Figure 3.24**) of the generated material showed no step-like feature prior to degradation. This was an expected outcome for an anhydrous sample.

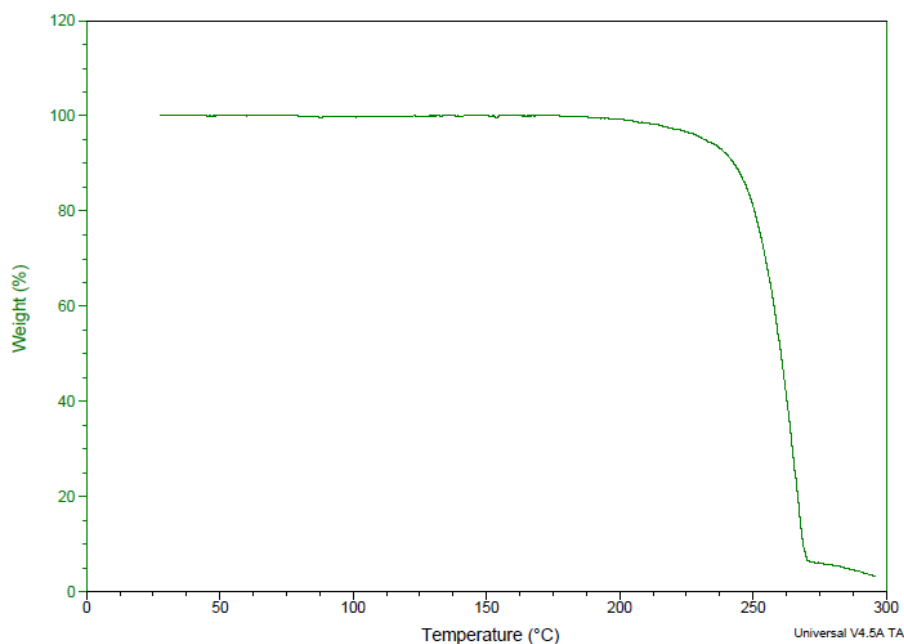


Figure 3.24: TGA graph of the sample crystallised in an attempt to prepare anhydrous CBZ, Form II.

3.3.2.3. Anhydrous Form IV

This polymorph was produced by means of dehydration of the dihydrate form under reduced pressure ^[75]. Additional details of the production method are provided in **Section 2.3.1.5.3**.

Microscope images of the resulting solid (**Figure 3.25**), showed differences to the Form III starting material (**Figure 3.1** (left)). While agglomerated clusters of the small irregular crystals of the starting material can still be seen, the presence of larger plate-like crystals is also seen, suggesting that a mixture of forms was present.

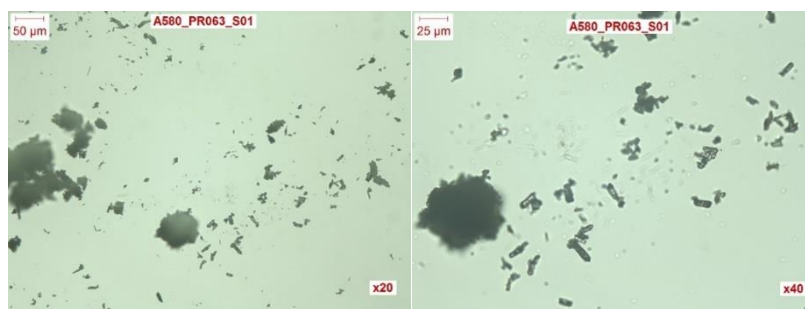


Figure 3.25: Light microscopy images of the sample generated in an attempt to prepare anhydrous CBZ, Form IV.

XRPD analysis of this sample was completed using the new XRPD available (**Section 2.4.5**). The XRPD pattern of the generated sample, when overlayed with the Form III anhydrous starting material (**Figure 3.26**), showed several similar peaks with both traces overlaying well. However, there were several other peaks which were only seen in the newly generated sample. These peaks, which were found at 16.44, 17.90, 21.30, 21.75, and 22.65° 2 θ , corresponded well to peaks for Form IV detailed in the literature [78]. This confirmed the presence of a mixture of polymorphs in this sample. Numerous attempts to generate Form IV alone consistently resulted in a mixture. The pressure levels achievable in the oven used might not be enough to drive a full conversion.

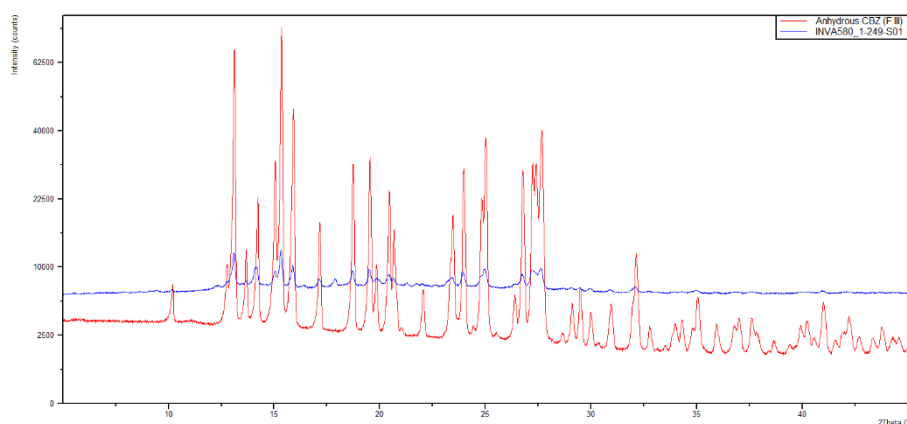


Figure 3.26: XRPD overlay of anhydrous CBZ, Form III (red) and the sample generated in an attempt to prepare anhydrous CBZ, Form IV (blue).

Due to a mixture of polymorphs being present, the expected DSC thermogram for Form IV was not obtained. Results for CBZ Form IV depend on the heating rate used. Here a 10 °C/min method was used (**Figure 3.27**). Based on results in literature ^[78], the analytical heating rate affects how the DSC trace of form IV behaves and based on the 10 °C/min scan rate used, two endotherms would be expected to be seen with a true Form IV sample – the first is believed to show melting and partial crystallisation of a new form between 178-187°C. ^[78] This event has a higher transition temperature than any seen in the DSC analysis of Form II and III. The second peak of Form IV results relates to the characteristic melt of the Form I polymorph (at 190-192 °C). However, experimental results don't show a melt of the Form IV polymorph but a shallow endotherm at ~160 °C which may relate to a transition of a different form as it is too low a temperature for Form IV. This is followed by the large, characteristic melt of Form I. The results achieved here compare well to that shown in **Figure 3.22**. Both experiments may have generated the same sample. TGA results were as expected showing only degradation, **Figure 3.28**.

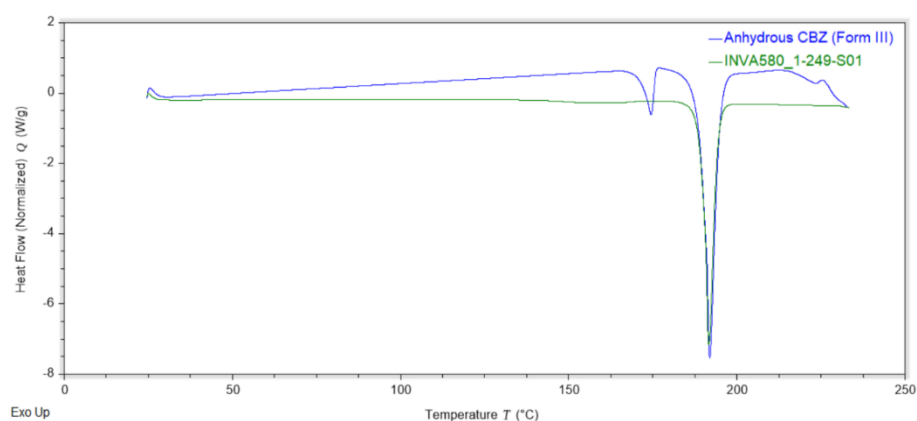


Figure 3.27: DSC thermogram overlay of anhydrous CBZ, Form III (blue) and the sample generated in an attempt to prepare anhydrous CBZ, Form IV (green).

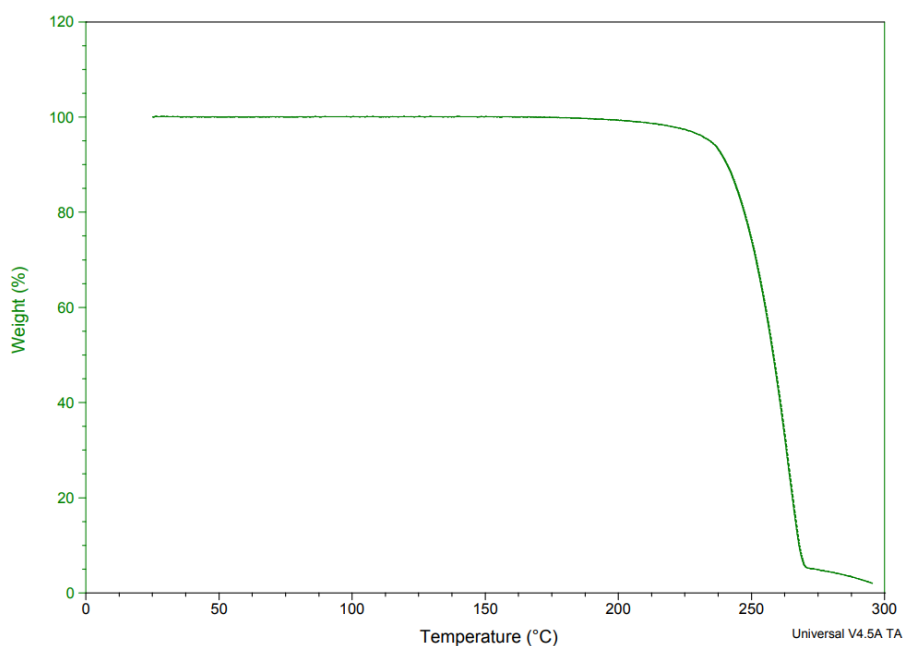


Figure 3.28: TGA graph of the sample generated in an attempt to prepare anhydrous CBZ, Form IV.

3.3.3. THEO Polymorphs

The generation of THEO polymorphs are detailed in **Section 2.3.1.9**. Solid-state analysis of each generated form was performed to further build out the library of results for future form identification.

3.3.3.1. Anhydrous Form I

A method from the literature was used ^[89] for the generation of the Form I polymorph (**Section 2.3.1.9.1**) from the monohydrate form. Several experiments were carried out, based on this method with the hope of finding the best way to efficiently heat the monohydrate sample – including using the EM System and flash heating using the Moisture Analyzer. In the end the Crystalline system was used as it gave more repeatable results.

Microscope images of the resulting solid (**Figure 3.29**), can be compared to both the Form III starting material (**Figure 3.10** (left)) and the generated monohydrate (**Figure 3.10** (right)). Clear

differences can be seen when compared to the monohydrate, with the long needle-like structures no longer present. The anhydrous Form I crystals are much smaller and show a tendency to agglomerate, which is similar to the Form II starting material. The smaller size is likely due to the agitation used within the experiment.

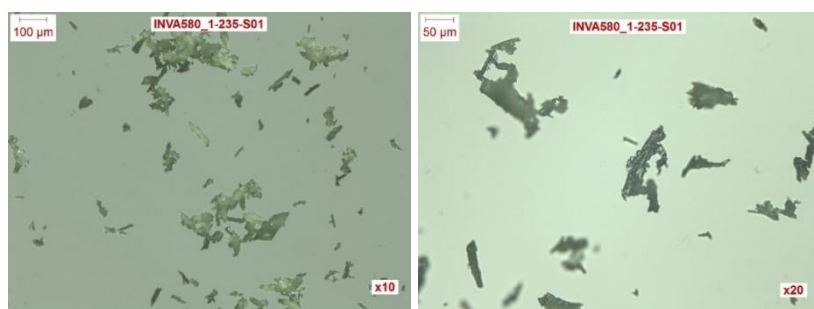


Figure 3.29: Light microscopy images of the sample generated in an attempt to prepare anhydrous THEO, Form I.

XRPD analysis of this sample was completed using the new XRPD available (**Section 2.4.5**). The XRPD pattern of the generated sample, was overlayed with both the Form II starting material and previously generated monohydrate and is shown in **Figure 3.30**. While resolution of the monohydrate and the newly generated sample is lower than that of the Form II starting material, due to sample size, XRPD shows that post heating of the monohydrate at 100 °C for 24 hr, there is a mixture of the Form II starting material and its hydrated form. Form I was not successfully generated.

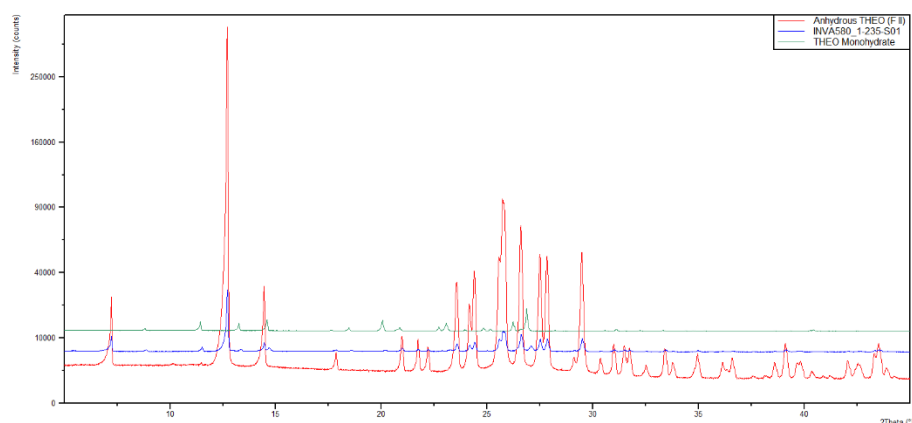


Figure 3.30: XRPD overlay of anhydrous THEO, Form II (red), sample generated in an attempt to prepare anhydrous THEO, Form I (blue) and THEO monohydrate (green).

DSC profiles (**Figure 3.31**), showed both thermograms with only one endotherm which had an onset temperature of ~ 272 °C. This corresponded to the characteristic melt of Form II. Coupling this with XRPD results, the presence of a peak for the vaporisation of water (relating to residual moisture of the monohydrate) was expected but was not present in either of the duplicate samples. Regardless of this, the desired form (Form I) was not present. TGA analysis showed an expected trace, with only degradation taking place as the temperature was increased, **Figure 3.32**.

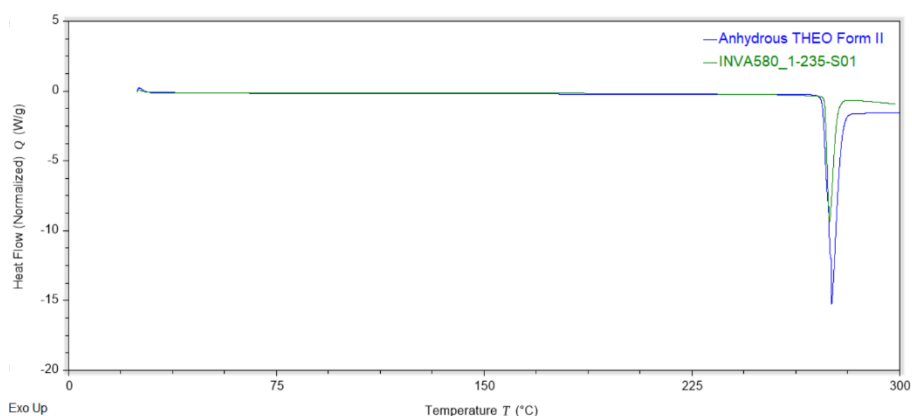


Figure 3.31: DSC thermogram overlay of anhydrous CBZ, Form II (blue) and sample generated in an attempt to prepare anhydrous THEO, Form I (green).

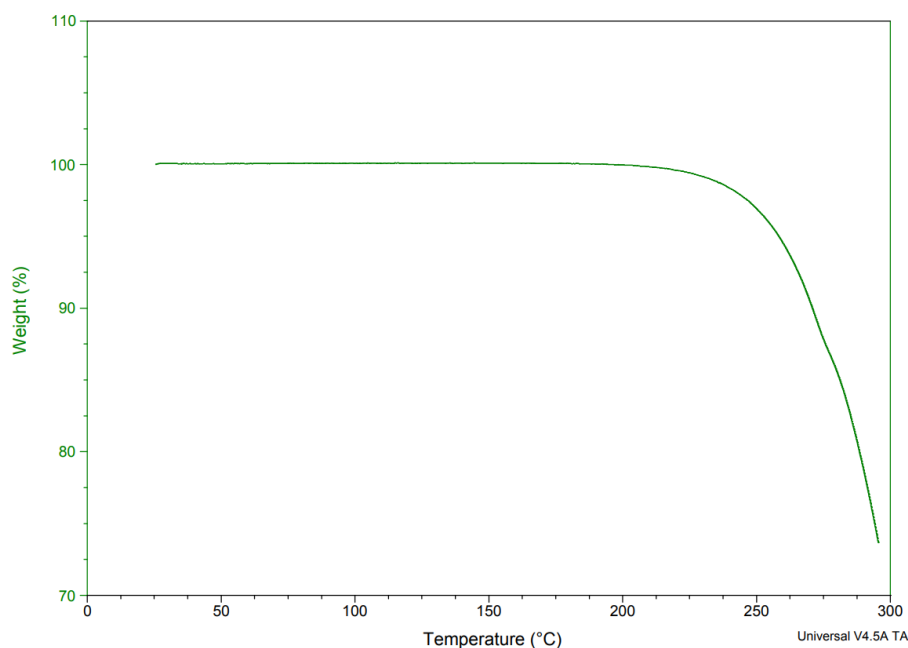


Figure 3.32: TGA analysis of sample generated in an attempt to prepare anhydrous THEO, Form I.

3.3.3.2. Anhydrous Form III

A method from literature was used ^[85] to inform the generation of the Form III polymorph by the dehydration of the monohydrate form at reduced pressures. Previously generated monohydrate was placed into a vacuum oven at 15 °C and at pressure < 200 mbar (**Section 2.3.1.9.2**). The sample was left at these conditions overnight and then analysed.

Microscope images of the resulting solid (**Figure 3.33**), can be compared to both the Form III starting material (**Figure 3.10** (left)) and the generated monohydrate (**Figure 3.10** (right)). Again, seeing clear differences are observed when compared to the monohydrate due to the dehydration process. Slight agglomeration of crystals was seen but the plate-like morphology was still present.

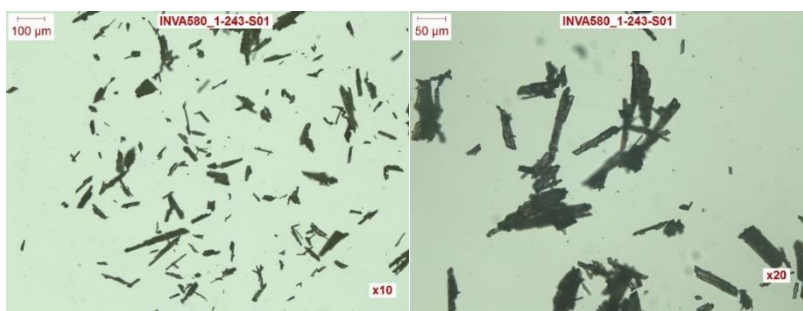


Figure 3.33: Light microscope images of anhydrous THEO, Form III.

XRPD results of both the generated solid and the Form II starting material are shown in **Figure 3.34**. Additional peaks were seen in the trace of the newly generated solid. Its XRPD trace compared very well to that of the metastable Form III trace presented in the literature [108].

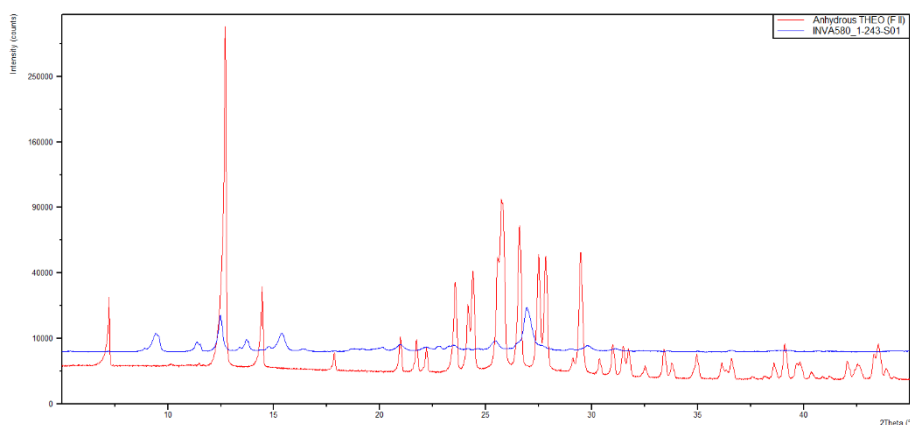


Figure 3.34: XRPD overlay of anhydrous THEO, Form II (red) and anhydrous THEO, Form III (blue).

DSC profiles (**Figure 3.35**) show one melting endotherm with an onset of ~ 272 °C, being the characteristic melt of Form II. In an effort to reduce its energy, Form III undergoes crystalline rearrangement to produce Form II. With a heating rate of 10 °C/min, no transition is seen. However, from literature [111], using higher heating rates, an exothermic transition corresponding to the change from Form III to Form II was clearly seen in the range of

160–200 °C. TGA analysis showed that once again degradation was the only feature present for this anhydrous polymorph, **Figure 3.36**.

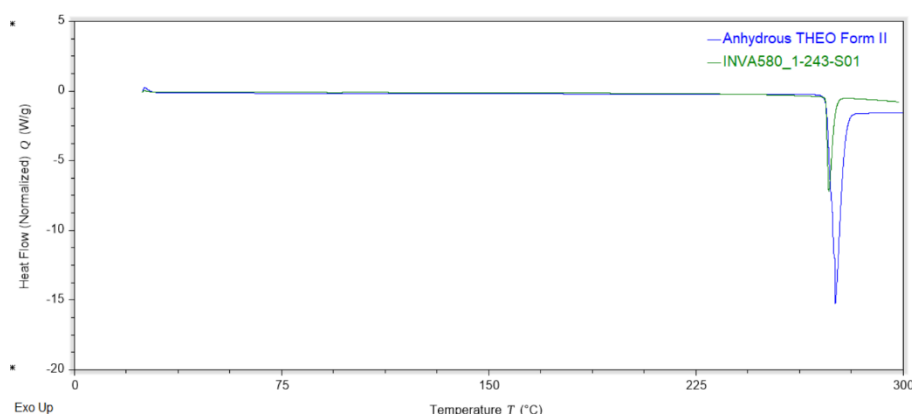


Figure 3.35: DSC thermogram overlay of anhydrous CBZ, Form II (blue) and anhydrous CBZ, Form III (green).

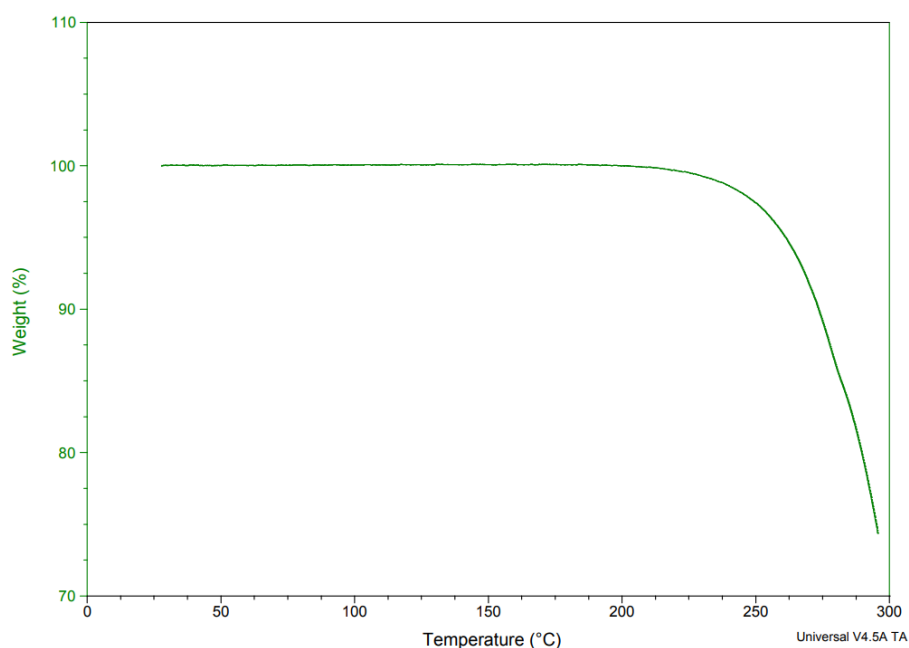


Figure 3.36: TGA analysis of anhydrous THEO, Form III.

3.3.3.3. Anhydrous Form IV

Initial attempts to generate the Form IV polymorph were made using a method which was cited numerous times ^[84], but with no success. A different method was found in the literature ^[86] that used a slightly different approach to the previously mentioned

method. In the second method, Form IV was prepared by suspending excess anhydrous Form II THEO in a MeOH-water mixture, whereby the volumes of MeOH and water used achieved a water activity value of <0.68 . Under these conditions, a transformation was reported to take place from Form I and II to Form IV within 2-30 days. However, in the presented work, the solid was left in solution for 35 days but Form IV was not generated.

Microscope (**Figure 3.37**) and photograph (**Figure 3.38**) images of the resulting solid after 33 days in suspension, show very large crystals. When compared to both the Form III starting material (**Figure 3.10** (left)) and the generated monohydrate (**Figure 3.10** (right)), the size difference is clear. When initially prepared, the slurry was sampled for microscopy to try and track the generation from rod-like structures to flat, plate-like structures of Form IV, based on results from literature.^[86] After 5 days, with no such transformation seen, the sample was left until after the 30-day mark. After this time, what remained were large, thick rod-like structures with no evidence of the hexagonal crystals.

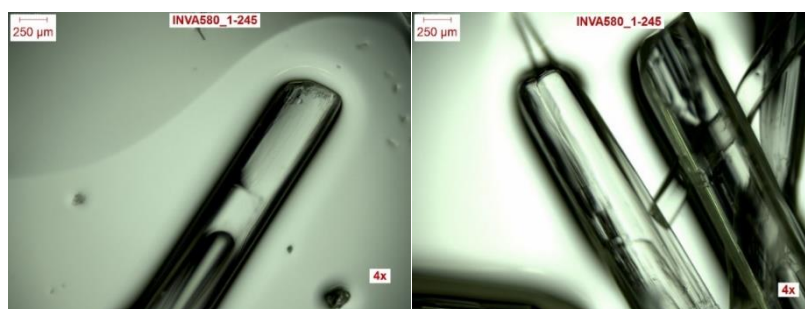


Figure 3.37: Light microscope images of crystals generated in an attempt to prepare anhydrous THEO, Form IV.

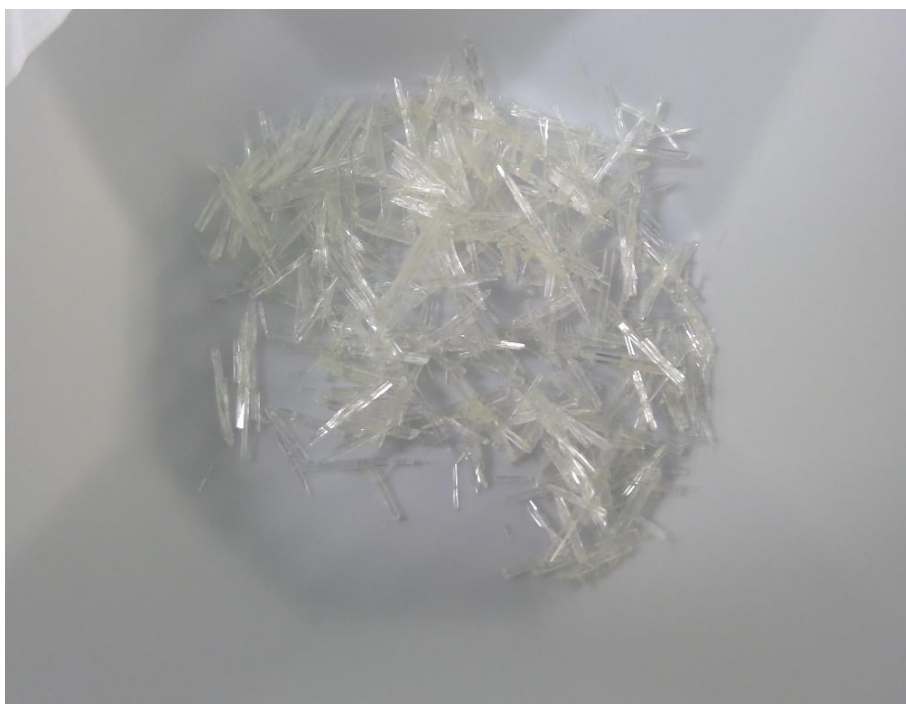


Figure 3.38: Photograph image of the large crystals generated in an attempt to prepare anhydrous THEO, Form IV.

XRPD results of the generated solid, Form II starting material and the monohydrate are shown in **Figure 3.39**. Form IV peaks as detailed literature ^[86] were not present and the diffractogram only showed a mixture of both the monohydrate and Form II starting material being present.

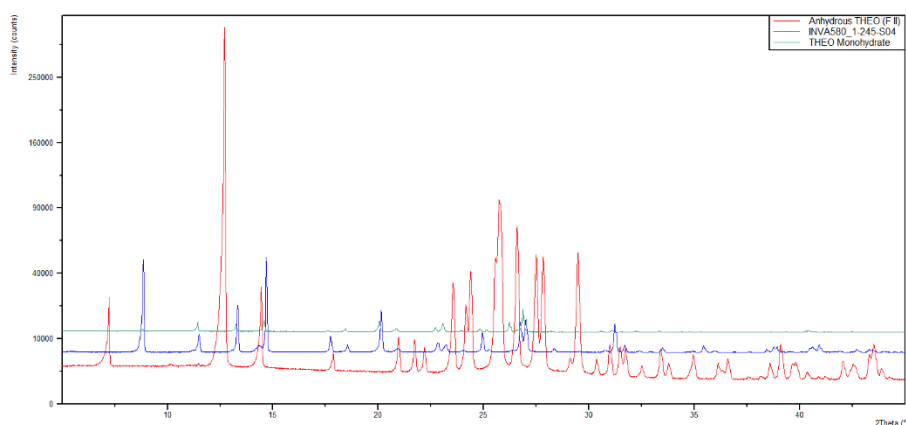


Figure 3.39: XRPD overlay of anhydrous THEO, Form II (red), sample generated in an attempt to prepare anhydrous THEO, Form IV (blue) and THEO monohydrate (green).

DSC profiles (**Figure 3.40**) of the generated solid show two endotherms. The first endotherm appears to relate to the loss of water from the monohydrate, the low onset temperature being indicative of the channel hydrate. The second endotherm at ~272 °C is the characteristic melt of Form II. TGA analysis (**Figure 3.41**) showed a step-like feature due to the loss of water which corresponded closely to the stoichiometric value for the monohydrate (9.08%). Based on the findings from the XRPD analysis, these results were expected.

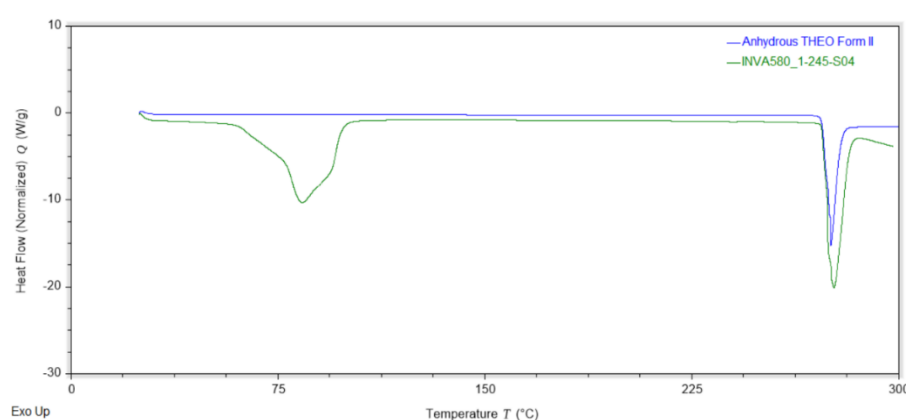


Figure 3.40: DSC thermogram overlay of anhydrous CBZ, Form II (blue) and sample generated in an attempt to prepare anhydrous THEO, Form IV (green).

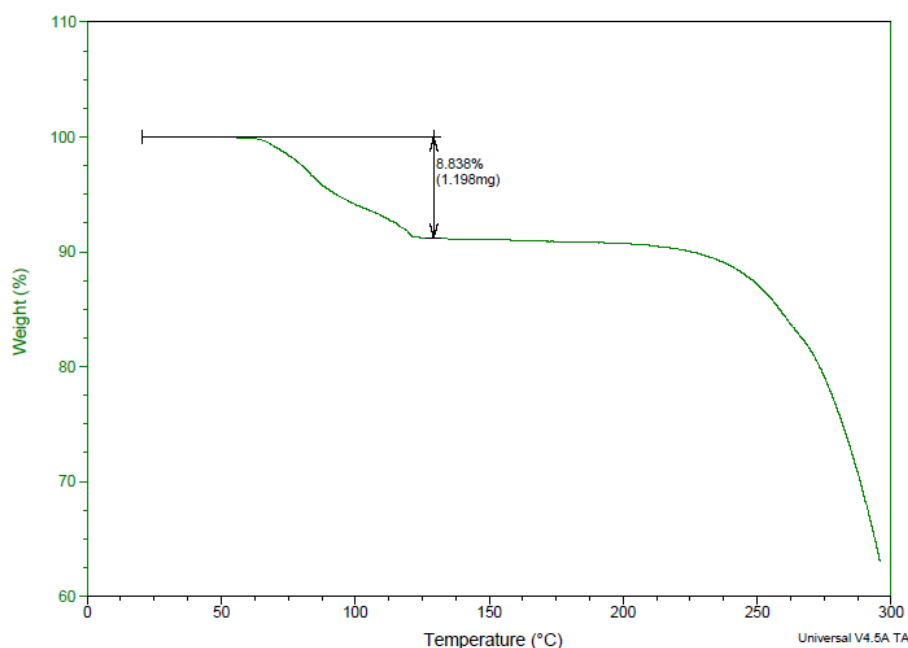


Figure 3.41: TGA analysis of sample generated in an attempt to prepare anhydrous THEO, Form IV.

3.3.4. Hydrate crystals with differing particle size

3.3.4.1. Solubility of the hydrate forms

Solubility is a thermodynamic process which describes the amount of solute that will dissolve in a pure solvent, at specific conditions. It is determined by intermolecular interactions in both the solute and solvent, which lead to cohesive and adhesive forces between both. Solubility is affected by many factors, including temperature which often has the greatest impact. It is commonly known that solubility increases with temperature. When water molecules heat up, they become more mobile and vibrate faster and will interact more readily and break apart the solute, meaning more sugar, for example, dissolves in warm water when compared to cold water.

Solubility data are used for making important decisions, even at the earliest stages of drug development. It is the first step in crystallisation process design. It can be done on a small scale, meaning minimal amount of sample is required while providing

valuable data about the solubility design space. Solubility data was gathered for both API compounds to inform crystallization methods which could be used to produce hydrate crystals of differing particle sizes.

3.3.4.1.1. CBZ Dihydrate Solubility

Due to the low solubility of CBZ dihydrate in water, a gravimetric-based equilibrium method was used to gather solubility data. A suspension of the solid was prepared and allowed to equilibrate at constant temperature. The resulting solution was then sampled as described in **Section 2.2.1.1**. Although a time-consuming approach, the gravimetric method is known to be very accurate. Other methods for gathering solubility data, like Crystal 16™ and using FBRM each involve turbidity measurements which proved difficult due to the material's low solubility in water. Turbidity measurements are made by determining the cloud and clear points of a solution, at increasing temperatures. Cloud points are where the crystals are in suspension whereas clear points show where the solids are dissolved. With low solubility, determining these clear points accurately is not possible.

For this work, it was important to ensure that the data being collected was of the dihydrate form itself. As discussed in **Section 3.3.1.1**, the conversion from the anhydrate to the hydrated form at room temperature starts instantly and within 25 min, only the needle-like structure of the dihydrate form was present. Coupling this with DSC results for the filtered solids (**Figure 3.42**), it can be deduced that the solubility data at 20, 40 and 60 °C was that of the hydrate form, whereas the DSC data for 80 °C showed a mixture of hydrate and anhydrous forms, after the solubility experiment. As a result, the solubility curve, **Figure 3.43**, only contains three data points.

Looking closer at **Figure 3.42** which shows the DSC thermograms of the solids for each of the triplicate experiments at 20, 40, 60 and 80 °C - for 20, 40 and 60 °C, the presence of the hydrate form was evident in each sample analysed. At 60 °C, the peaks of hydration had lower onset temperatures when compared to the samples at 20 and 40 °C suggesting that the water in these samples was being removed more easily. The peaks of hydration at 60 °C showed additional differences to one another, with the third sample having a much sharper peak when compared to the first sample which had a broader peak. This could be due to sample preparation. For an ideal DSC sample, the pan should have a very thin layer of sample across the bottom of the pan to ensure uniform heat transfer. Due to agglomeration after filtering and ambient drying this was difficult to achieve and could have resulted in increased cake depth within the sample pan which then affected the heat transfer. Excess sample leads to inefficient heat transfer and can cause a broader peak. For the 80 °C samples, there were differences seen across triplicate samples. Only one of the samples had a thermogram resembling the hydrate form. The other two thermograms show CBZ Form III. At this temperature, there are changes occurring and the gravimetric solubility data collected, at this set of conditions, is not of the dihydrate form, hence why the fourth data point is not included in the solubility curve (**Figure 3.43**).

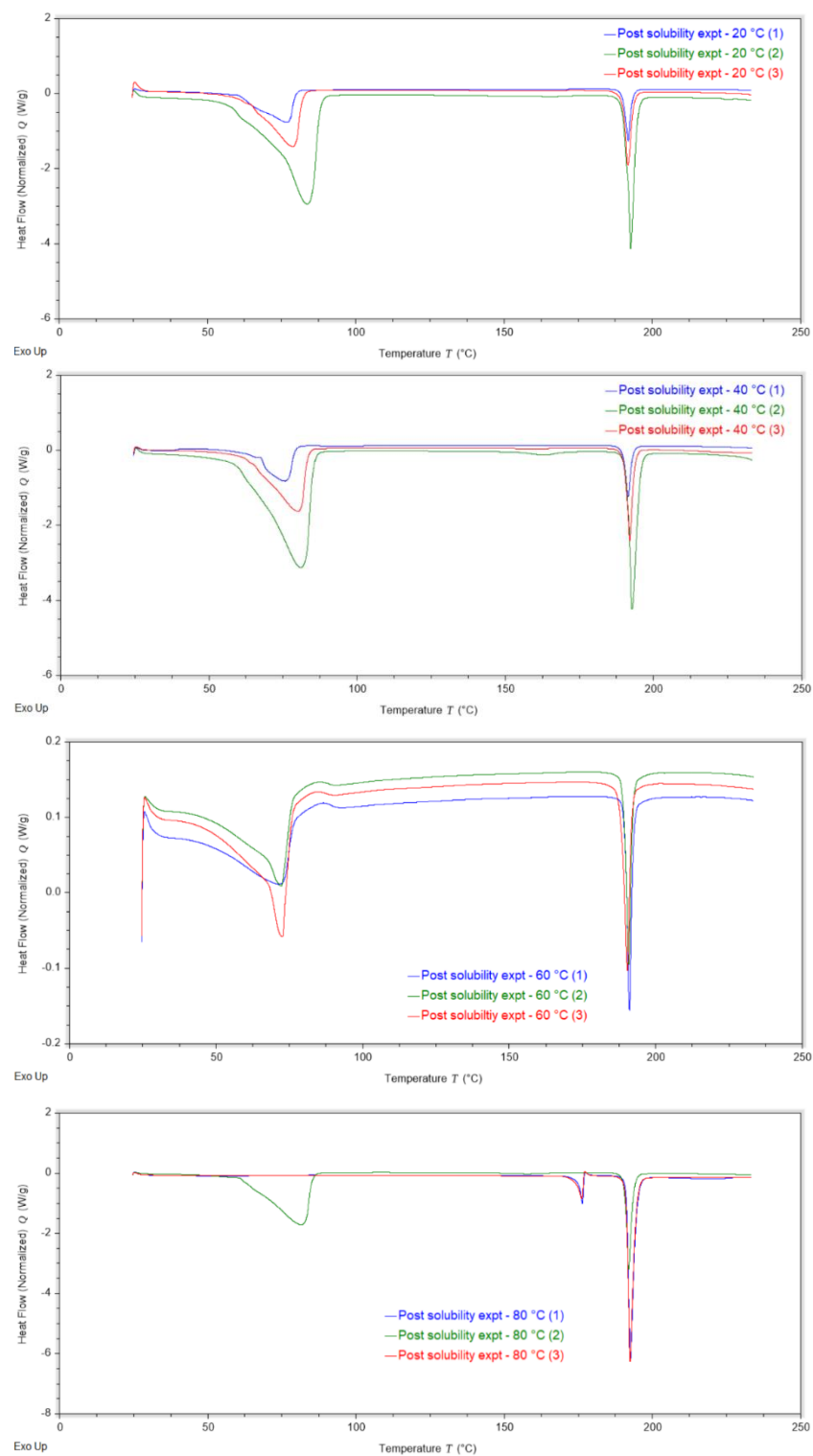


Figure 3.42: DSC traces of excess solids recovered by filtration from solubility experiments (in triplicate) at 20 °C, 40 °C, 60 °C and 80 °C.

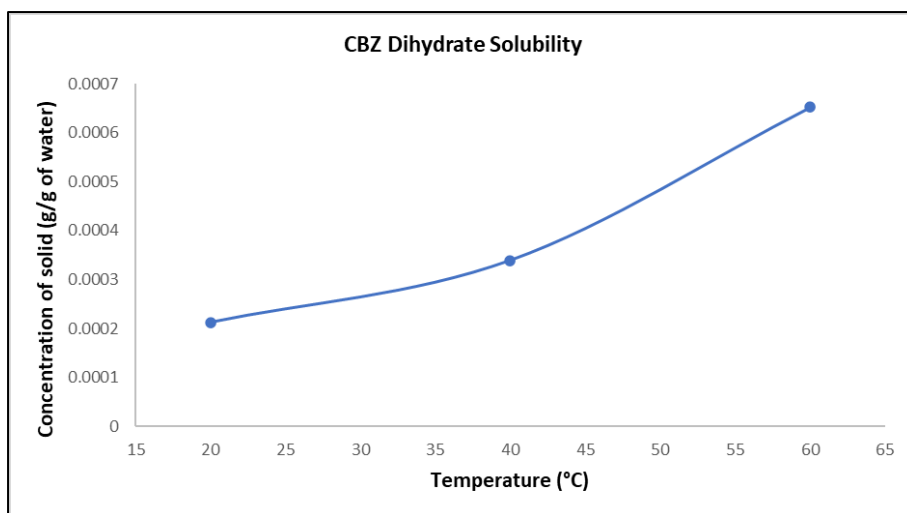


Figure 3.43: Solubility curve of CBZ dihydrate in water.

3.3.4.1.2. THEO Monohydrate Solubility

THEO is more soluble in water than CBZ, therefore Crystal16™ could be used for determining THEO monohydrate solubility data. Coupling THEO's better solubility with a temperature variation method, previously discussed (**Section 3.3.4.1.1**), cloud and clear points could be determined. For this work, previously generated monohydrate solid was used in these experiments. A five-point solubility curve was successfully generated (**Figure 3.44**).

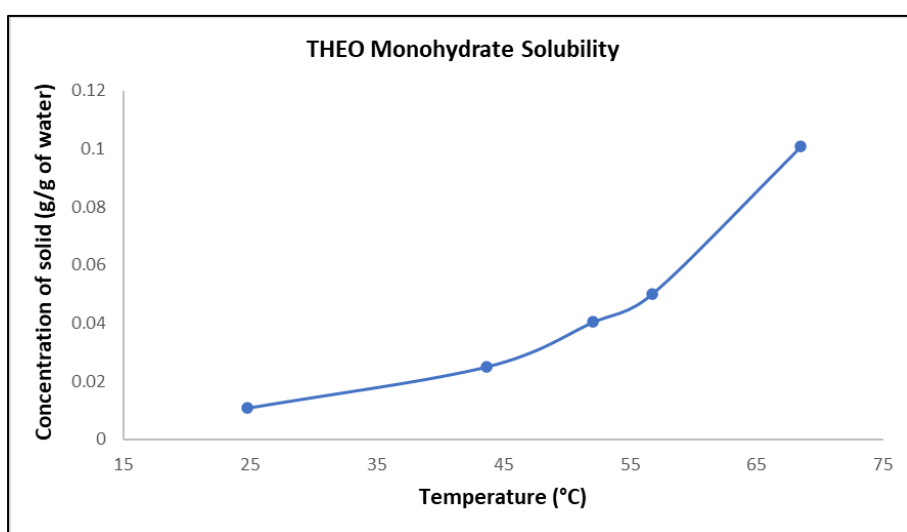


Figure 3.44: Solubility curve of THEO monohydrate in water.

Another advantage of using Crystal16™ is that by cycling temperatures, additional information can be gathered on clear and cloud points, and this can inform the Metastable Zone Width (MSZW), which is of interest in designing robust crystallisation processes should this information be required.

3.3.4.2. CBZ Dihydrate – Differing Particle Sizes

The generation of hydrate crystals with differing PD was investigated with the intention of using the resulting solids in a later Stability Study (**CHAPTER 4**) and a Drying Study via DVS (**CHAPTER 5**).

A steep solubility curve would suggest that a small change in temperature would cause a large increase in supersaturation. With this, there is then the potential for fast and potentially uncontrolled nucleation or growth. However, CBZ dihydrate has a gentle solubility curve (low slope) (**Figure 3.43**), meaning changes in temperature need to be greater to see the increase in supersaturation needed for crystallisation to occur. Initial attempts to use a cooling crystallisation method with a fast-cooling rate to generate small crystals of CBZ dihydrate were unsuccessful. Differences in particle size, when compared to “Medium” crystals (generated by the standard method already) were not statistically significant. Poorly soluble compounds are often downsized via milling, whether wet or dry, to facilitate a reduction in PSD and, in turn, an improvement in dissolution and bioavailability. ^[112] Based on this, a wet milling method was seen as a suitable alternative for the reduction in particle size when these unsuccessful crystallisation attempts arose. A thermocycling method was also employed. For this method, solubility data and the post solubility DSC traces were coupled together to inform the parameters. DSC traces of the 80 °C post solubility experiment (**Figure 3.42**), showed

a mixture of forms being present which made 80 °C an unusable upper temperature for the temperature cycling method. Solubility over 20-60 °C was very low and, as previously discussed (**Section 3.3.4.1.1**), cloud and clear points were difficult to obtain. However, to maximise the temperature difference (to increase supersaturation based on the gentle solubility curve) it was decided to use an upper temperature of 70 °C for the dissolution of smaller crystals and by reducing the temperature, encourage solute to precipitate on the surface of existing crystals. Solid-state analysis, post thermocycling proved the dihydrate form was the only form present and that the higher temperature of 70 °C did not have negative impacts.

Informed by the solubility data and supporting analysis of the dihydrate, three different methods were used to generate CBZ dihydrate crystals of varying particle size:

- Small particles: crystals were generated using the standard method (**Section 2.3.1.2**) and then wet milled to reduce particle size (**Section 2.3.1.3.1**). Milling involves applying mechanical energy to coarse particles and physically breaking them down, resulting in finer particles. This is considered a ‘top-down’ approach for the production of finer, smaller particles ^[113].
- Medium particles: the previously discussed standard method was used (**Section 2.3.1.2**).
- Large particles: temperature cycling method was used to grow/ripen the dihydrate crystals (**Section 2.3.1.3.3**). The principle of temperature cycling involves the manipulation of temperatures for the dissolution of crystals in undersaturated conditions and the growth/ripening of remaining crystals in a supersaturated solution ^[114].

The main objective for this work was to produce three batches of hydrate crystals with differing particle size. To confirm whether these methods were successful, particle size was measured. DSC and XRPD was also undertaken to ensure the hydrate form was not jeopardised by any of the methods used.

Microscope images (**Figure 3.45**) showed that the needle-like morphology which is characteristics of CBZ dihydrate was consistently found in each of the samples. Changes in particle size are difficult to discern from these images alone. Laser diffraction (Malvern 3000) was used to determine the PSD.



Figure 3.45: Light microscopy images of CBZ dihydrate of differing particle sizes: Small crystals (left), Medium crystals (middle) and Large crystals (right).

PSD profiles determined using the Malvern 3000 (**Section 2.4.6**) showed shifts to the right as we move from Small to Large particles (**Figure 3.46**). This shift is confirmed by the data gathered for the d10 and d50 values. Examining the d50 values of each sample, it shows a progression in size from $\sim 12\ \mu\text{m}$, to $\sim 18\ \mu\text{m}$ and finally, $\sim 39\ \mu\text{m}$. Variations in d90 values were evident.

CBZ dihydrate became electrostatic after isolation, which made sampling difficult. Alongside this, CBZ dihydrate formed hard agglomerates upon drying under ambient conditions. As detailed in **Section 2.4.6**, the sample was sonicated in the dispersion unit of the particle sizer for 10 sec at 80% and then allowed to circulate for 10 min prior to measurement. This was to encourage de-

agglomeration and helped to tighten the % RSD for replicate measurements.

Despite the differences in d90 values, the differences in crystal sizes, as indicated by the d10 and d50 values, were deemed sufficient for additional work - a stability study which would investigate the effects of temperature and % RH on hydrate stability/dehydration of CBZ dihydrate material with different particle sizes (**CHAPTER 4**) and for assessing the impact of particle size on drying and dehydration during the Drying Study via DVS (**CHAPTER 5**).

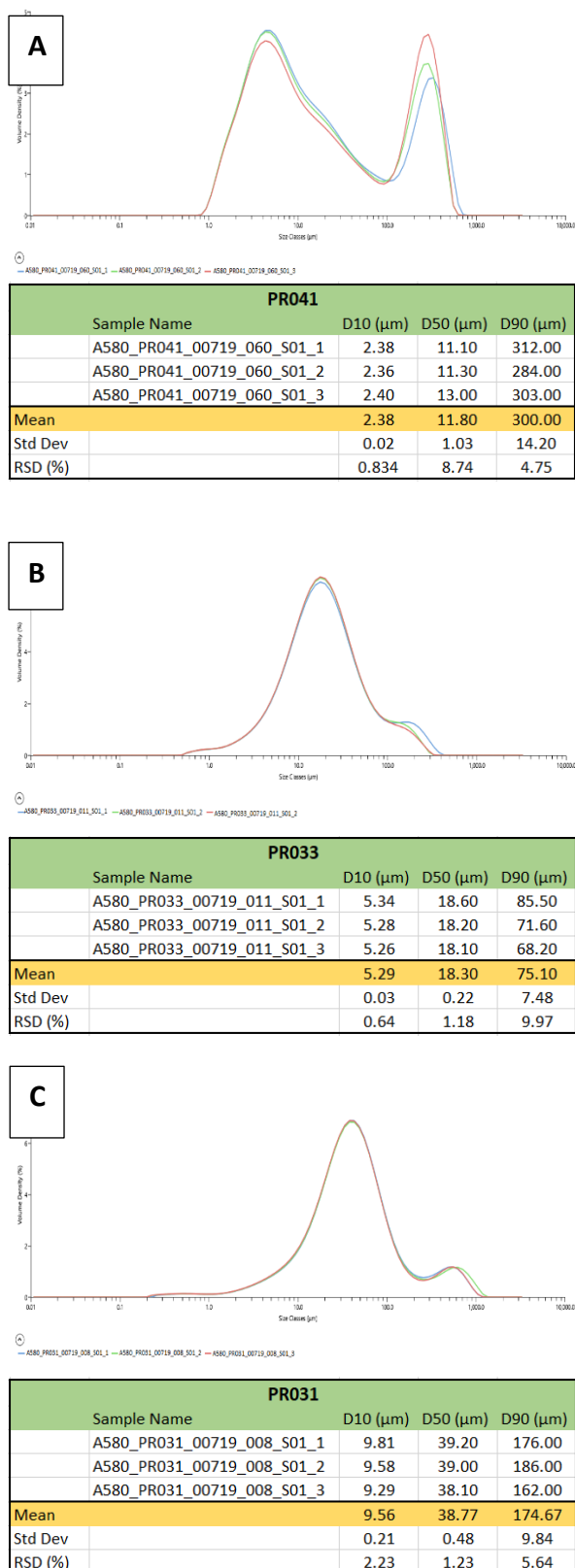


Figure 3.46: PSD analysis of CBZ dihydrate particles; Small crystals (top), Medium crystals (middle) and Large crystals (bottom).

After particle size changes were confirmed, DSC analysis was used to investigate possible changes to the solid-state form due to the mechanical forces of milling or temperature cycling on the dihydrate form. From **Figure 3.47**, peaks of hydration were present in each of the three samples, along with the characteristic Form I melt at $\sim 190^\circ\text{C}$. The peaks of hydration had small differences in onset temperatures, from $\sim 71^\circ\text{C}$, $\sim 60^\circ\text{C}$ and $\sim 63^\circ\text{C}$ for Small, Medium and Large crystals respectively. Enthalpy values for the peak of hydration decreased across the samples from Small to Large; 343.6 J/g, 256.28 J/g, 143.09 J/g. Vaporisation is the breaking of intermolecular bonds. From these values it appears that the bonds in the Large crystals take less energy to be broken and for the water of hydration to convert to a vapour.

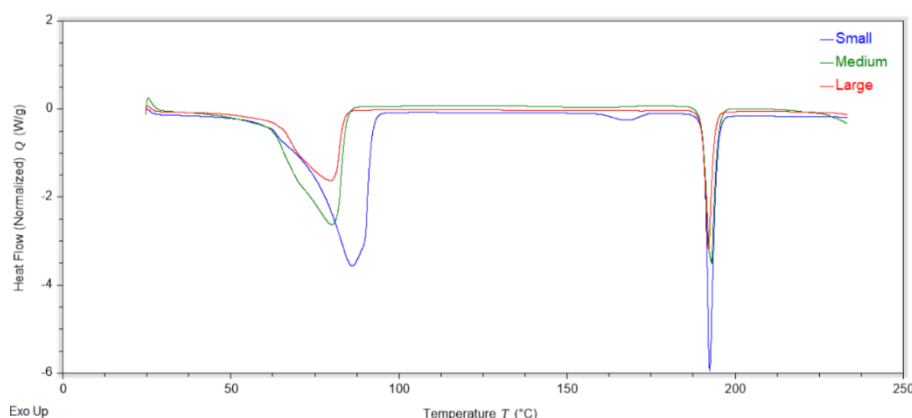


Figure 3.47: DSC overlay of CBZ dihydrate samples of differing particle size.

Finally, to confirm that the dihydrate is present after milling and temperature cycling, XRPD analysis was carried out (**Figure 3.48**). All samples overlaid well and all characteristic peaks of the dihydrate form were present. Neither milling nor temperature cycling had adverse effects on the dihydrate form.

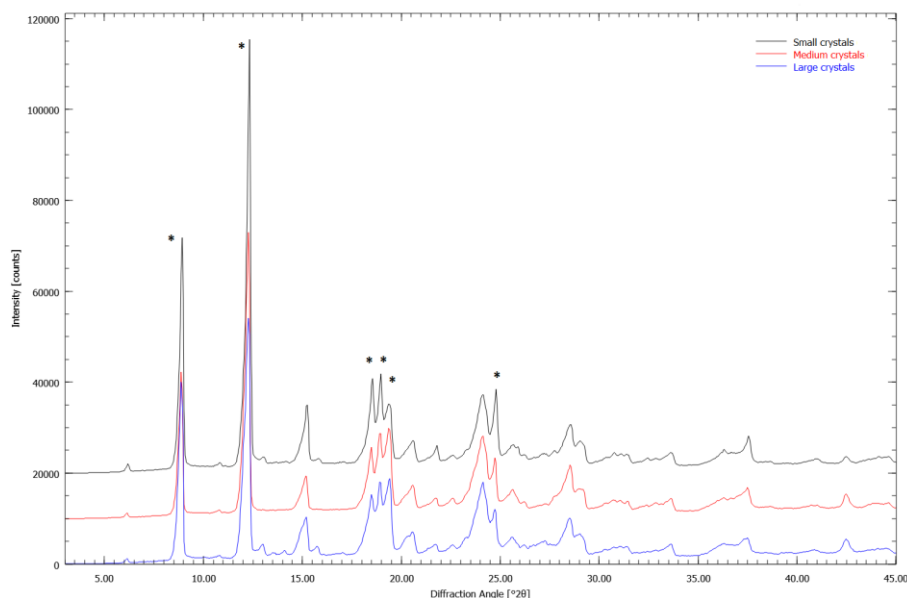


Figure 3.48: XRPD overlay of CBZ dihydrate of differing particle sizes. * denotes the characteristic peaks of the dihydrate.

In summary, for CBZ dihydrate, milling successfully downsized hydrate particles and temperature cycling successfully grew and ripened larger hydrate particles.

3.3.4.3. THEO Monohydrate – Differing Particle Sizes

The generation of hydrate crystals with differing PSD was carried out with the intention of using the resulting solids in a later Stability Study (**CHAPTER 4**) and Drying Study via DVS (**CHAPTER 5**).

As a cooling crystallization method was used as the standard method for generating THEO monohydrate, it was decided to explore other cooling rates for generating crystals of different sizes. Cooling within a crystallisation method causes the solution to become supersaturated and by altering the rate of cooling, PSD can be altered. Fast cooling rates give rise to small crystals as there is minimal time for the crystals to be formed. The opposite is true with slow cooling rates. When given a longer time to form, crystals grow and become large. Another major advantage of using cooling

crystallizations is high uniformity of crystal size in the product at a set cooling rate. The sharp decrease in solubility for THEO monohydrate with decreasing temperatures (**Figure 3.44**) also supports using cooling crystallization methods.

By using different cooling rates, differences in PSD were achieved:

- Small particles: the method detailed in **Section 2.3.1.7.2** was used but with an increased cooling rate of 1 K/min. By increasing the cooling rate, the highest level of supersaturation is reached. As a result, the system is driven by fast nucleation rather than crystal growth.
- Medium particles: the standard method detailed in **Section 2.3.1.7.2** was used.
- Large particles: the method detailed in **Section 2.3.1.7.2** was used but with a slower cooling rate of 0.1 K/min.

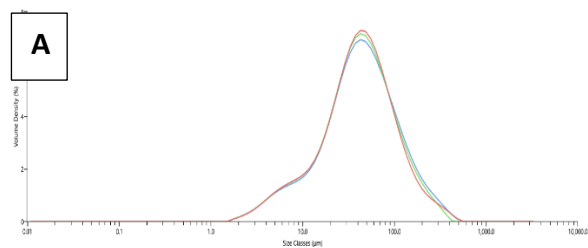
Microscope analysis (Figure 1.30) showed that the crystal morphology stayed consistent across all three samples with plate-like crystals present. When examining the image of the 'large' crystals, an increase in crystal size appears to have occurred but more detailed, quantitative analysis of PSD was required to confirm this.



Figure 3.49: Light microscopy images of THEO monohydrate of differing particles sizes; Small crystals (left), Medium crystals (middle) and Large crystals (right).

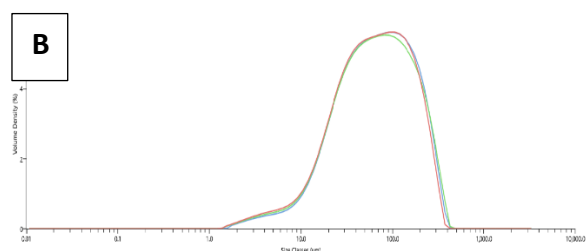
Malvern 3000 analysis confirmed that the particle size increased when the cooling rate was decreased. THEO monohydrate was easy to work with and produced a non-static, free-flowing powder meaning additional method development to ensure de-agglomeration for PSD analysis was not required, and the % RSD for all sample measurements was very low.

Figure 3.50 shows the distributions for each sample along with the d10, d50 and d90 values. The distributions show a shift to the right, with PSD values confirming the particle growth. Comparison of all values across the three samples showed successful crystal growth with a decreased cooling rate.



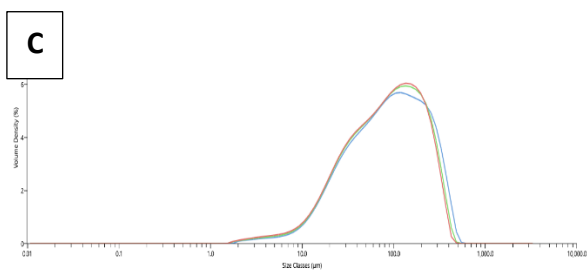
—A580_PR039_00719_056_S01_1 —A580_PR039_00719_056_S01_2 —A580_PR039_00719_056_S01_3

PR039				
Sample Name	D10 (µm)	D50 (µm)	D90 (µm)	
A580_PR039_00719_056_S01_1	10.20	42.80	137.00	
A580_PR039_00719_056_S01_2	9.91	41.60	126.00	
A580_PR039_00719_056_S01_3	9.90	41.60	123.00	
Mean	10.00	42.00	128.67	
Std Dev	0.14	0.57	6.02	
RSD (%)	1.39	1.35	4.68	



—A580_PR040_00719_052_S01_1 —A580_PR040_00719_052_S01_2 —A580_PR040_00719_052_S01_3

PR040				
Sample Name	D10 (µm)	D50 (µm)	D90 (µm)	
A580_PR040_00719_052_S01_1	17.00	66.60	208.00	
A580_PR040_00719_052_S01_2	16.30	65.40	212.00	
A580_PR040_00719_052_S01_3	15.60	63.30	197.00	
Mean	16.30	65.10	205.67	
Std Dev	0.57	1.36	6.34	
RSD (%)	3.51	2.09	3.08	



—A580_PR037_00719_051_S01_1 —A580_PR037_00719_051_S01_2 —A580_PR037_00719_051_S01_3

PR037				
Sample Name	D10 (µm)	D50 (µm)	D90 (µm)	
A580_PR037_00719_051_S01_1	21.00	89.80	276.00	
A580_PR037_00719_051_S01_2	20.00	86.00	251.00	
A580_PR037_00719_051_S01_3	19.30	83.80	241.00	
Mean	20.10	86.53	256.00	
Std Dev	0.70	2.48	14.72	
RSD (%)	3.47	2.86	5.75	

Figure 3.50: PSD analysis of THEO monohydrate; Small crystals (top), Medium crystals (middle) and Large crystals (bottom).

The DSC traces (**Figure 3.51**) show that each sample compared well, and the monohydrate was present. No additional endothermic or exothermic peaks were introduced by changes to the cooling rate. Onset temperatures had only minimal changes for the peak of hydration, and the characteristic Form II melt at ~ 275 °C was seen in each of the samples. XRPD patterns (**Figure 3.52**) show that each sample contains the characteristic peaks of the monohydrate and overlay well. However, for the 'medium' particles, an additional peak was present at ~ 12.5 ° 2θ (highlighted in the graph with an arrow) which corresponds to a peak found in the starting material, anhydrous Form II polymorph, indicating that a small amount of the anhydrate form was present in this sample. Quantitative analysis was not completed to determine relative amounts of the anhydrous material.

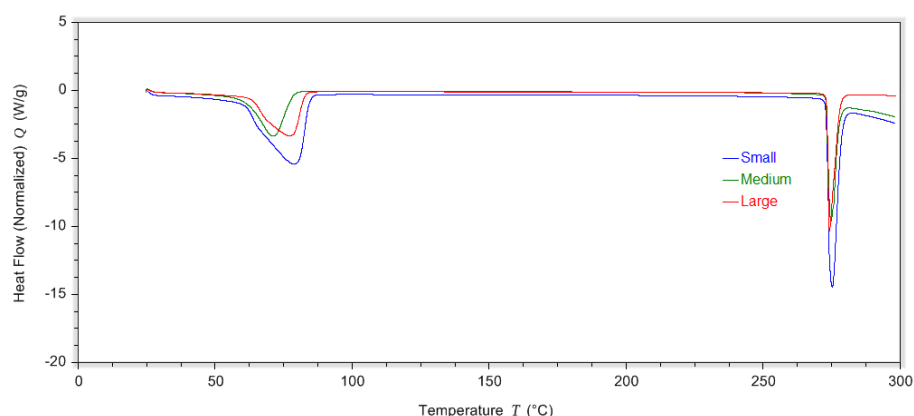


Figure 3.51: DSC trace overlay of CBZ dihydrate of differing particle sizes.

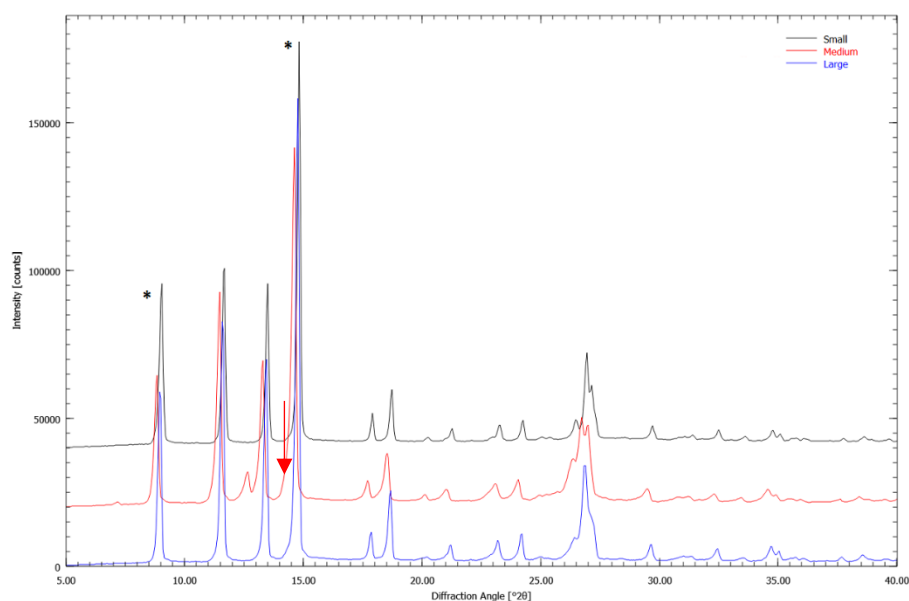


Figure 3.52: XRPD overlay of THEO monohydrate; Small crystals (black), Medium crystals (red) and Large crystals blue). * denotes the characteristic peaks of the monohydrate. The red arrow shows a peak characteristic of THEO anhydrous Form II polymorph.

Unfortunately, this XRPD sample was not examined prior to using the samples for additional work. When the PSD analysis and DSC analysis all came back successful, a close examination of the XRPD patterns was overlooked. The next body of work involved using these solids in a stability study and several chambers were placed in their specific environments, prior to noting this extra peak. It was decided to continue despite this. This work will be discussed in detail in the next chapter.

3.4. Chapter Conclusions

A library of solid-state results was successfully gathered for both compounds (CBZ and THEO), their hydrate forms, and polymorphs.

Solubility of CBZ dihydrate in water was gathered using a gravimetric method and data showed the hydrate form was poorly soluble in water. CBZ dihydrate was successfully generated using a slurry conversion method with DIW, at room temperature. When

tracked with PVM, the conversion appears to occur quickly when the anhydrous form is added to water and nears completion within the first hour. For CBZ's polymorphs, methods of production ranged from crystallisations from ethanol for Form II and direct heating of a solid with agitation for Form I. Due to unsuccessful attempts to generate Form IV from an ethanol crystallisation process, Form IV was not generated. For crystals with differing PSD, milling was used in the downsizing of CBZ dihydrate. Standard crystals were first generated, and then mechanical energy was used to reduce the PSD while keeping the hydrate form intact. Despite CBZ dihydrate having low solubility, generating Large crystals was achieved using a temperature cycling method, by using an upper temperature which did not adversely affect the hydrate form (<80 °C). This allowed for small crystals to dissolve and, when the system was cooled, precipitation of solute onto existing hydrate crystals occurred. Confirmation of increasing PSD values was completed by laser diffraction analysis.

For THEO monohydrate, solubility data was collected using Crystal 16 [™] as cloud and clear points were easy to determine. THEO monohydrate had a higher solubility when compared to CBZ dihydrate. THEO monohydrate itself was generated using a cooling crystallisation method in water. Cooling crystallisation methods proved to be the method of choice for THEO monohydrate crystals, both for the robust generation of monohydrate crystals but also in the production of hydrate crystals of differing particle size. Morphological changes between the anhydrous and monohydrate form were more difficult to discern from microscopy and SEM analysis, however additional solid-state analysis confirmed its conversion to the hydrate form and for the presence of the different polymorphic forms. Using literature, methods for the generation of each polymorphic form were gathered and executed

in order to build a library of results. This library would then be used to help aid form identification after drying and/or dehydration carried out in the Stability Study (**CHAPTER 4**) and the Drying Study via DVS (**CHAPTER 5**).

For crystals of differing sizes, PSD values for d10, d50 and d90 increased across all samples when the cooling rate was decreased, with the monohydrate confirmed to be present in each sample also.

From this work, robust methods for generation of hydrate forms for batches of sample throughout the project was achieved. Through the successful generation of crystals with differing sizes, examining the effects of micromeritic properties on hydrate stability and drying could now be investigated.

CHAPTER 4. Stability Study on hydrate forms of both model compounds using the Cuspor Aging System

4.1. Abstract

The aim of this work was to test the time taken for hydrates of CBZ and THEO to lose water of hydration and show evidence of a form change to an anhydrous or dehydrated form, when placed in a specific controlled temperature and humidity environment.

An initial stability experiment found that when exposed to 0% RH at 20 °C, it took 33 days for CBZ dihydrate to lose its water of hydration, while for THEO monohydrate, under the same conditions, it took 13 days to see the loss of the water of hydration. XRPD analysis of end point samples (where there was no step change in the TGA indicative of water of hydration) indicated that both hydrated samples had transformed back to the original anhydrous starting materials from which the hydrate crystals were prepared. However, in each case there was one additional peak in the XRPD pattern which corresponded to a peak seen in the diffractograms of the hydrate form. These findings were the starting point for undertaking a larger scale hydrate stability study.

The subsequent stability study was carried out temperatures of 25, 50 and 80 °C and at % RH of 2, 40 and 75% RH. A cross section of conditions were decided on and, for both CBZ dihydrate and THEO monohydrate, evidence of hydrate stability was only found under one set of conditions: 75% RH and 25 °C. Under these conditions, for both compounds, hydrate stability was seen over a 12-week period. For all other samples, at other conditions, the hydrate forms were negatively impacted by their environments which ultimately resulted in the loss of the hydrated forms.

Samples of THEO monohydrate with different particle size distributions showed no differences in dehydration behaviour under different stability conditions. However, for CBZ dihydrate at centre point conditions (of 40% RH and 50 °C), TGA values for 'Large' crystals took longer to reach a weight loss of 0.00% in the TGA - indicating loss of the dihydrate form - than the 'Small' and 'Medium' crystals. Also, in conditions where the dihydrate was still present (based on TGA values) after 12 weeks, XRPD results showed 'Small' and 'Large' crystals showing initial signs of transitioning back to the CBZ anhydrous starting material.

Stability study results demonstrated the sensitivity of the hydrate forms of both CBZ and THEO to dehydration, with high humidity (75%) and moderate temperature (25 °C) being the only condition of those tested under which hydrate solid state stability could be maintained on storage.

4.2. Introduction

Stability studies play a vital role in estimating shelf lives and providing information on storage conditions, based on stress testing products under controlled environmental conditions such as humidity, temperature, and light. Prior to investigating the drying of hydrated crystals and determining the time point, under specific drying conditions, where a change from removal of loosely bound moisture to water of hydration occurs, understanding the boundaries of each hydrate's stability was deemed important. This was evaluated using the same principles as a stability study, under controlled environmental conditions. By finding the limits, beyond which the water of hydration is negatively impacted, future drying studies would come from a place of knowledge and lead to more targeted experimental plans.

Stability of any hydrated drug product is strongly influenced by dehydration which may lead to the formation of a lower hydrated state, dehydrated hydrate, amorphous material, and/or an anhydrous form. ^[115] Consequently, it is crucial for drug development to be able to predict the dehydration of crystalline hydrate based on the intermolecular interactions of the water molecules present in the crystals. ^[37] Will the loss of water through dehydration cause the collapse of the crystal lattice or, based on the interactions of the water molecules and the host molecules, can the lattice withstand the removal of the water? Dehydration can be caused by incorrect storage conditions, processing, packaging and over drying. The work in this chapter details the stability of the hydrated forms of both CBZ and THEO and the relationships between the hydrated and dehydrated/anhydrous forms of both model compounds by examining the time and conditions under which dehydration begins and how long it takes for the water of hydration to be removed.

An initial stability experiment was set up with both hydrated forms of the model compounds, CBZ dihydrate and THEO monohydrate. The aim was to test the time taken for the endotherm on a DSC thermogram corresponding to removal of the water of hydration to disappear when placed in a specific controlled environment. Portions of CBZ dihydrate and THEO monohydrate were placed into separate glass crystallisation dishes. These were placed inside an incubator at 20 °C and 0% RH. The 0% RH environment was achieved using phosphorous pentoxide. Samples were taken at specific time points and subjected to DSC analysis to track the reduction in the dehydration endotherm. Once the DSC thermogram resembled that of an anhydrous sample, additional XRPD analysis was carried out to confirm the form of the end point

sample. The findings from this experiment led to an extended study to give further insights into the stability of both hydrates.

This aim of this larger stability study was to initially investigate the effects of temperature and % RH on hydrate stability and to do this efficiently, the Cuspor Ageing System, see **Figure 2.4**, was decided upon as an appropriate setup. The Cuspor setup provided a closed environment of desired conditions which minimised fears of cross contamination when using communal ovens and incubators. Another advantage of the Cuspor setup was the ability to stack sample vials when using the shallower option for glass sample vials. These vials could be stacked and separated by the vial platform which ensured the opening of each vial was exposed. With this option, it was decided to extend the initial investigation to also examine the effects of particle size on hydrate stability. Previous research ^[116] has demonstrated that particle size is important in determining the transformation kinetics of dehydration processes. Understanding how hydrate stability is affected by micromeritic properties such as PSD will ultimately inform robust drying protocols for both compounds.

4.3. Results and Discussion

4.3.1. Initial Stability Experiments

4.3.1.1. CBZ Dihydrate

Prior to the stability experiment, the generated sample (**Section 2.3.1.2**) was analysed by DSC, TGA and XRPD to confirm the presence of the dihydrate. **Figure 4.1 (A)** shows the DSC thermogram which contains the broad endotherm characteristic of the water of hydration, followed by a smaller endotherm of the Form III melt and then a recrystallisation exotherm for the Form I polymorph. The large endotherm at ~190 °C is the characteristic melt of Form I. TGA analysis (**Figure 4.1 (B)**) shows a weight loss step of 12.94% (no standard deviation value available as sample was only analysed once). This value equates closely to 13.22% which is the theoretical value for the dihydrate. The XRPD pattern (**Figure 4.1 (C)**) for the CBZ dihydrate sample used in this experiment contains all the peaks characteristic of the dihydrate and overlays well with other previously generated dihydrate batches. After this off-line analysis confirmed the sample to be the dihydrate form it was then prepared for the stability experiment (**Section 2.7.1**).

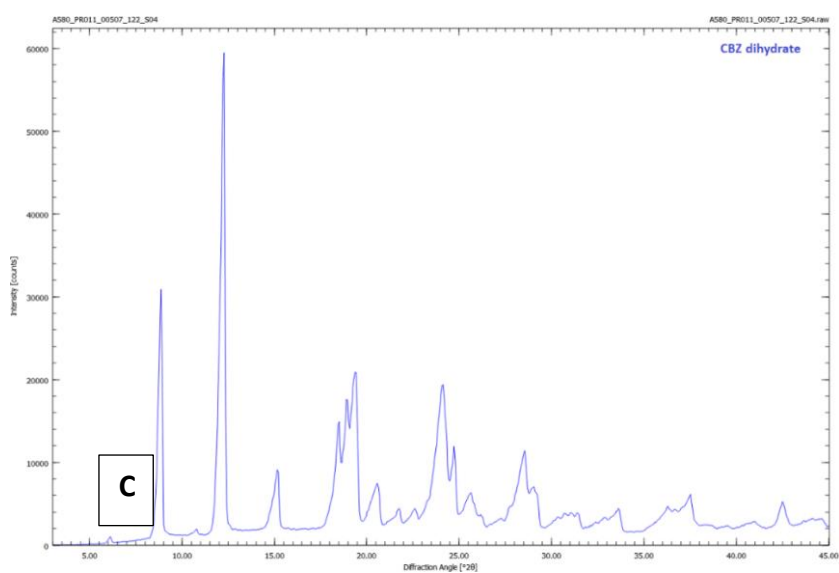
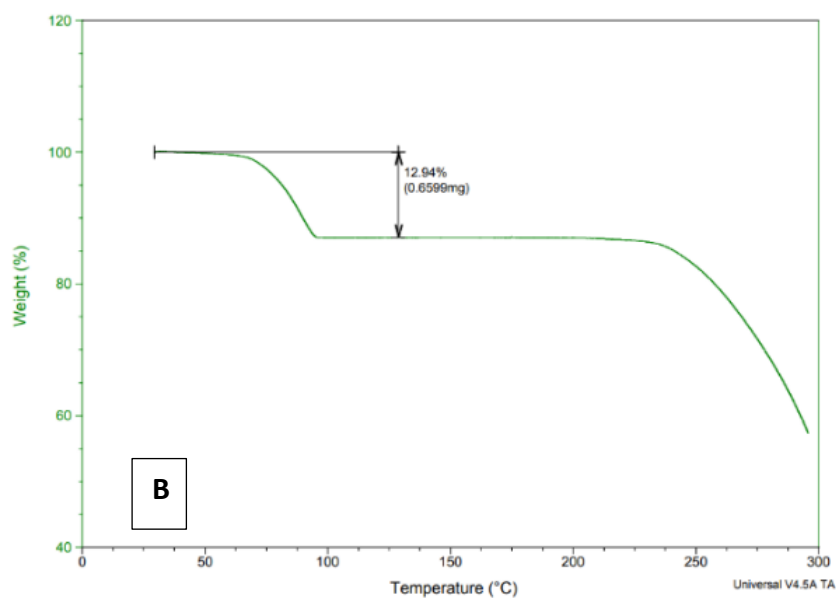
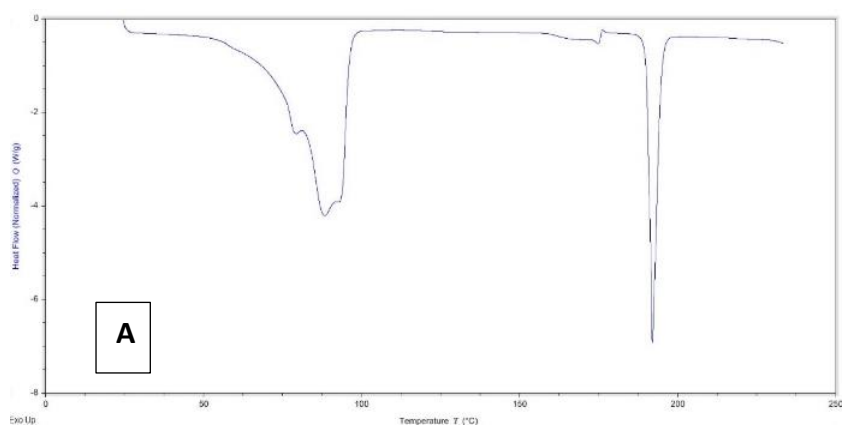


Figure 4.1: (A) DSC thermogram, (B) TGA and (C) XRPD pattern for CBZ dihydrate sample that would be subject to stability study.

When CBZ dihydrate was exposed to 0% RH at 20 °C, it took 33 days for the peak of hydration to disappear on the DSC thermogram (**Figure 4.2**). Along with this, the smaller endotherm indicative of the Form III melt disappeared. The final DSC trace (day 33) compares well with anhydrous Form III CBZ.

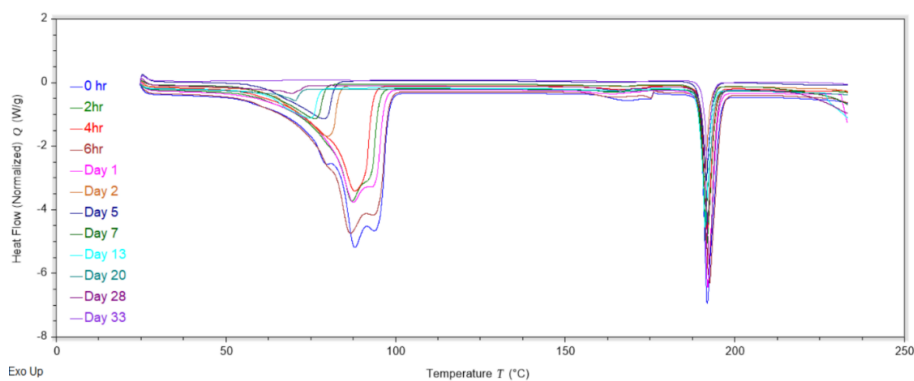


Figure 4.2. DSC overlay for CBZ dihydrate over time when exposed to 0% RH at 20 °C.

After day 33, XRPD, TGA and DVS analysis was carried out to confirm the form of the end point sample. TGA showed no step-like features (**Figure 4.3**) unlike the starting sample (**Figure 4.1 (B)**) which indicated that the hydrate was no longer present. XRPD analysis showed a pattern which overlaid with the starting material (anhydrous Form III CBZ), except for one peak at $\sim 12^\circ 2\theta$, which matched the hydrate pattern (**Figure 4.4**). This raised the question of whether the sample was, in fact, an anhydrous form or a dehydrated hydrate which differed structurally from the anhydrous form, showing a different XRPD pattern to both the hydrate and anhydrous starting materials.

DVS analysis gave further insight into the new (day 33) solid. Before the stability study, a sorption value (at 90% RH) of 13.46% was determined (**Figure 4.5 (left)**) and afterwards, it was 0.27% (**Figure 4.5 (right)**). By comparing the isotherms in **Figure 4.5**, the post-experiment sample is shown to be less stable. The desorption

isotherm showed a gradual loss in moisture at each step, unlike the initial hydrate sample which remained stable until 40% RH during desorption. Prior to stepping through levels of % RH, both methods begin with a drying step, at 0% RH for 600 min. This removes all the moisture from the sample and as neither sample is hygroscopic, the increase in mass with the increase in % RH levels relates to the level of hydration for the samples. The level of hydration (monohydrate, dihydrate etc) can be determined through calculations based on the compound's molecular weight.

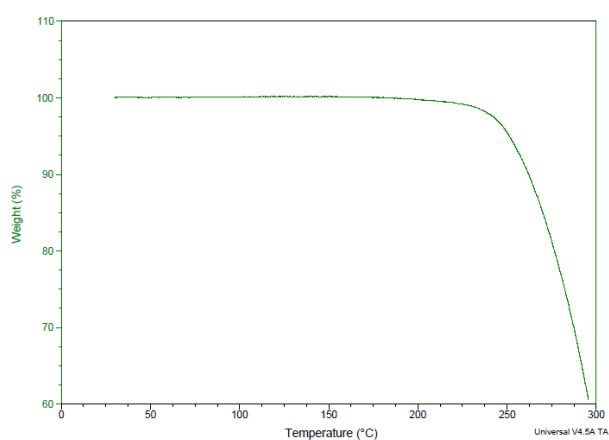


Figure 4.3: TGA analysis for a CBZ sample post 33 days at 0% RH and 20 °C.

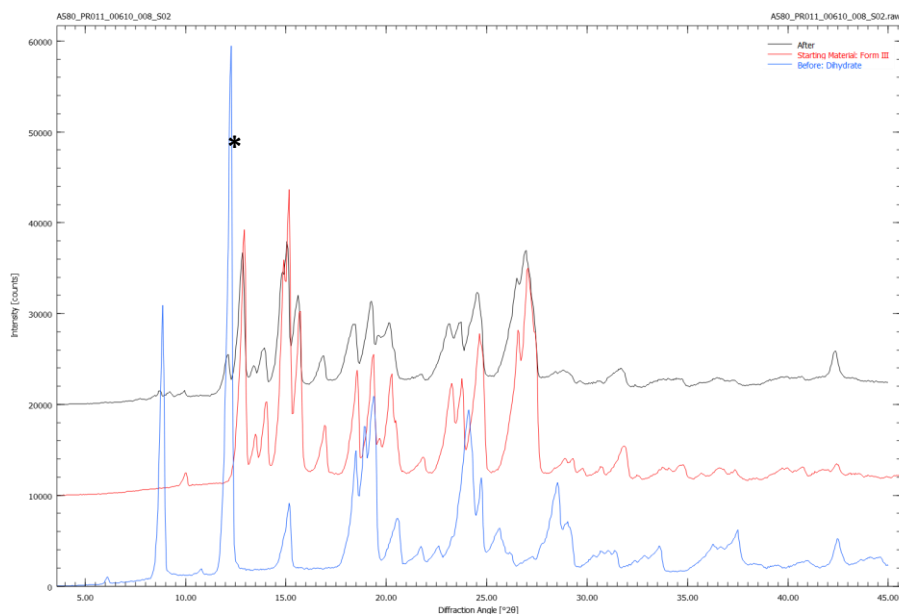


Figure 4.4: XRPD overlay for anhydrous Form III CBZ (red), pre-stability experiment dihydrate sample (blue) and post-stability experiment sample (33 days; 0% RH at 20 °C) (black).

The asterisk indicates a peak characteristic of the dihydrate, and which was found in the post stability experiment sample.

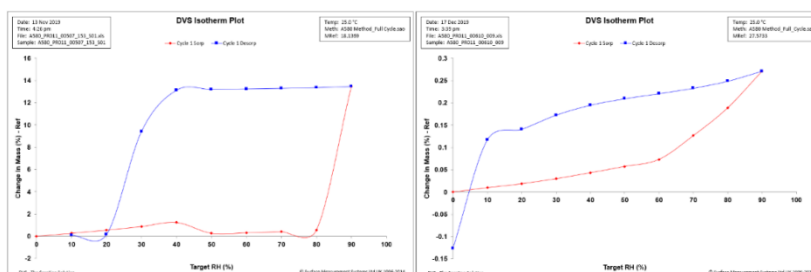


Figure 4.5: DVS analysis of CBZ-DH pre- (left) and post- (33 days; right) stability experiment (0% RH at 20 °C).

4.3.1.2. THEO Monohydrate

Prior to the experiment, the generated sample (preparation details **Section 2.3.1.6**) was analysed by DSC, TGA and XRPD to confirm the presence of the monohydrate. **Figure 4.6 (A)** shows the DSC thermogram which contains the broad endotherm characteristic of the water of hydration, followed by the characteristic Form II

melting endotherm at ~275 °C. TGA analysis (**Figure 4.6 (B)**) shows a weight loss step of 9.085% (no standard deviation value available as sample was only analysed once). This value equates closely to 9.08% which is the theoretical value for the monohydrate. The XRPD pattern (**Figure 4.6 (C)**) for THEO monohydrate used in this experiment contains all peaks characteristic of the monohydrate and overlays well with other previously generated monohydrate batches.

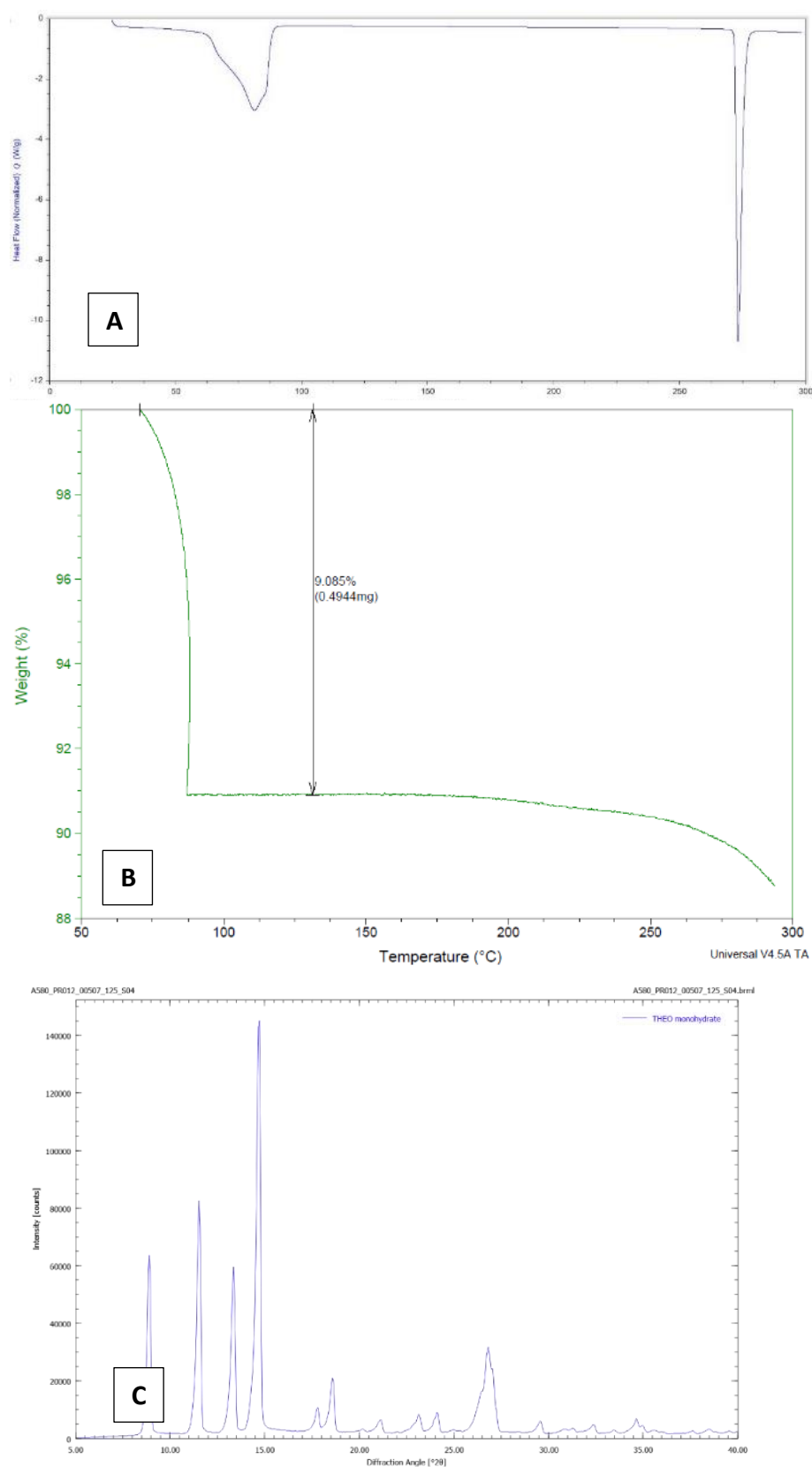


Figure 4.6: (A) DSC thermogram, (B) TGA and (C) XRPD pattern for THEO monohydrate sample that would be subject to stability study.

When THEO monohydrate was exposed to 0% RH at 20 °C, it took 13 days for the peak of hydration on the DSC trace to disappear (**Figure 4.7**). The shouldering seen in the peak of hydration is likely due to a build-up of pressure within the pan due to a lack of pinholes in the sample pans. The importance of the pinholes was noted during the experiment, but it was decided to continue with the same sample preparation for comparative purposes. TGA analysis (**Figure 4.8**) of the solid retrieved after 13 days showed no weight loss prior to degradation, showing no presence of the hydrate form. XRPD analysis (**Figure 4.9**) showed that the post-experiment sample overlaid well with anhydrous THEO, except for one peak at 11.5° 2 θ . This peak was present in the pre-experiment hydrate sample.

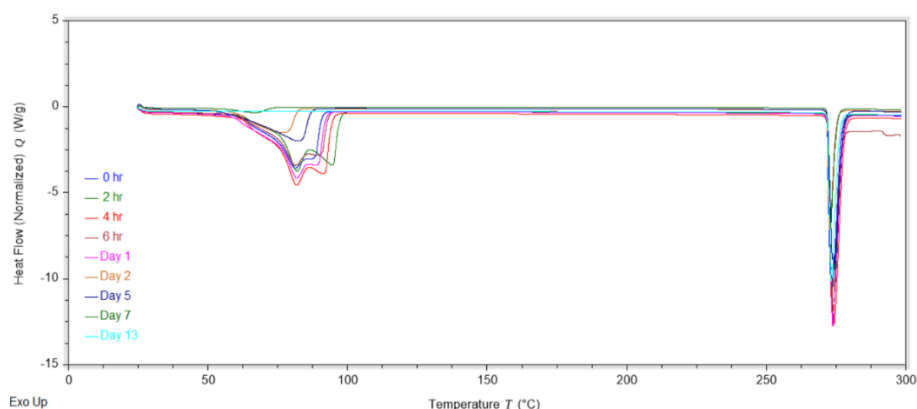


Figure 4.7: DSC overlay for THEO monohydrate over time when exposed to 0% RH at 20 °C.

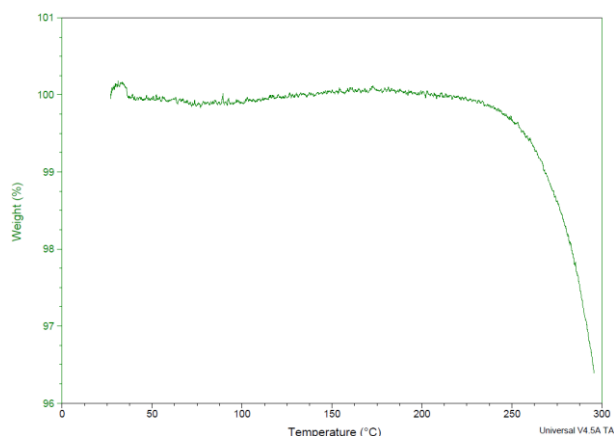


Figure 4.8: TGA analysis for THEO monohydrate after being exposed to 0% RH at 20 °C.

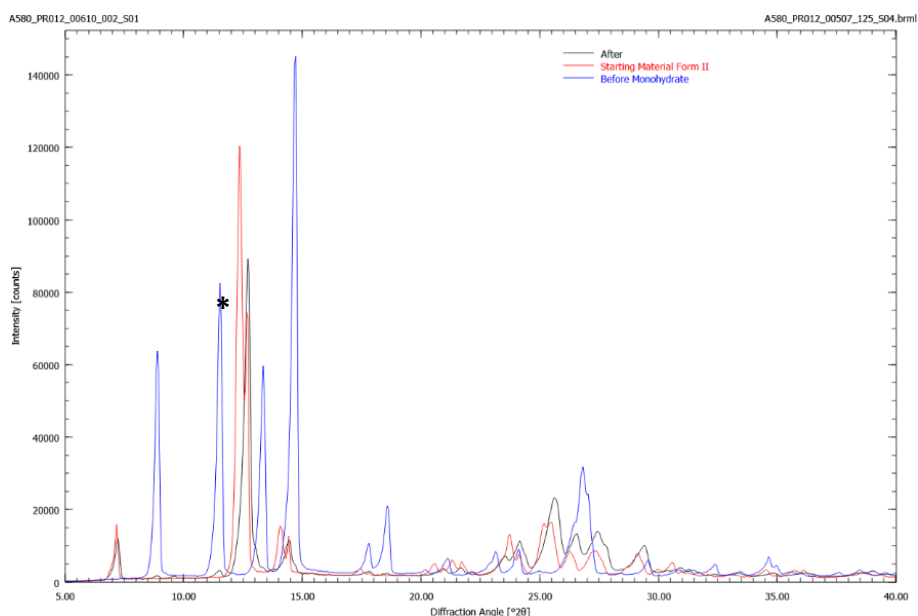


Figure 4.9: XRPD overlay of the THEO anhydrous (red), pre-stability experiment THEO monohydrate sample (blue) and post-stability experiment sample (13 days; 0% RH at 20 °C) (black).

The asterisk indicates a peak characteristic of the monohydrate, and which was found in the post stability experiment sample.

Figure 4.10 shows the DVS analysis of the THEO monohydrate before and after the stability experiment. There is a clear difference between the two samples. In contrast to CBZ dihydrate, the hysteresis increased for the THEO sample placed on stability.

Sorption values went from 9.13 % to 10 % and the resulting solid showed an increase in stability on the desorption profile. Hydrate stability of the sample in the DVS before the 13-day stability experiment (based on the desorption profile) was evident in environments of 90-50% RH, whereas afterwards, stability was evident from 90-40% RH.

The method used for each of the DVS experiments involves an industry standard, 10 hr drying step at 0% RH. This step dehydrates the sample prior to increasing the % RH environments so that the true uptake of moisture can be determined. The original THEO monohydrate sample (**Figure 4.10 (left)**), shows an anomalous loss of mass between 40-50% RH in the sorption profile. Mass loss coupled with an increase in RH levels is typical for the crystallisation of an amorphous material^[110], as water is expelled from the structure upon crystallisation. However, the sample was believed to be crystalline from the start. This calls into question the nature of the sample post the 10 hr drying step. A dehydrated form is expected to contain characteristics of both the stable anhydrous form and the hydrate form, with spaces where the water molecules exited. This is often referred to as a 'ghost hydrate structure'.^[110] It is possible that some disorder due to differences in crystal packing arises with the initial 10 hr 0% RH step, and that subsequent crystallisation/re-ordering arises with moisture sorption in the DVS. This feature is not seen on the sorption isotherm of the sample which was subjected to 0% RH for 13 days in the Cuspor unit. For this sample (**Figure 4.10 (right)**), there is no change in sample weight until a critical RH is reached, 60% RH. Previous work^[110] detailed that the change in mass between 40 and 50% RH was only seen during the rehydration of the dehydrated form (post 10 hr at 0% RH step) and not seen during an initial hydration of the THEO lattice. This appears to be the case here also.

The sample shown in **Figure 4.10** (left) began as the monohydrate, had all water removed during the 10 hr DVS drying step and was subsequently rehydrated, giving rise to the anomalous mass change, whereas the material shown in **Figure 4.10** (right), starts as either a dehydrated or anhydrous form (with dehydration/transition taking place over 13 days outside the DVS). With no water present, the 10 hr DVS drying step does not dry the sample any further. It is possible that either the 13 days under phosphorous pentoxide conditions or the additional hold for 10 hr gave the sample more time to re-order its crystal packing so that this anomalous loss of mass was not present during the sample's rehydration.

From the post-experiment solid-state analysis, TGA showed no presence of the hydrate form. However, the DVS data showed the sample held on stability (for 13 days) to have a reversible hydrate formation tendency, with the sorption-desorption profiles indicating that water both entered and exited the crystal lattice through channels which were strong enough to remain intact when the water was initially removed under stability study conditions.

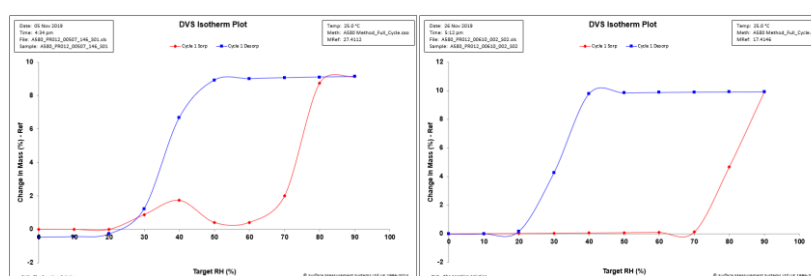


Figure 4.10: DVS analysis of THEO monohydrate pre (left) and post (13 days; right) initial stability experiment (0% RH at 20 °C).

These initial experiments provided interesting results; solid state and thermal analysis of CBZ and THEO hydrate forms indicated they behaved similarly under 0% RH conditions, although time to DSC-evidenced dehydration differed, while the two compounds also

showed differences in their before and after DVS analysis behaviour.

After this preliminary stability study, it was decided to carry out a larger stability study which would look at a range of temperatures and different % RH conditions. Along with those parameters it was decided to also look at the effect of particle size under specific conditions. Particles which were to be analysed were classified as: Small, Medium and Large (as detailed in **Sections 3.3.4.2** and **3.3.4.3**). A comparison of the water content and solid-state characteristics of the sample pre- and post- stability study would give an insight into the hydrate stability under various conditions, while also indicating boundaries beyond which the hydrate form cannot be protected. Knowing these boundaries would inform future drying studies.

4.3.2. Hydrate Stability Study – CBZ Dihydrate

As discussed in **Section 2.7**, this work was completed using the Cuspor Ageing System (**Figure 2.4**). This setup allows for samples to be held in airtight, % RH controlled environments using saturated salt solutions or desiccant and with temperature being controlled externally by ovens or incubators. To examine a wide range of conditions in an efficient manner, a cross section of available conditions was chosen. **Table 4.1** below shows this cross section.

Table 4.1: Hydrate stability study conditions for CBZ dihydrate.

CBZ Dihydrate			
	25 °C	50 °C	80 °C
2 %RH	Vial 1-A		Vial 1-C
40 %RH		Vial 2-B	
75 %RH	Vial 3-A		Vial 3-C

The effects of PSD on hydrate stability were also investigated. Each large outer Cuspor vial contained three samples of CBZ dihydrate of differing particle size, each in a separate smaller vial stacked one on top of the other. For each vial, the stacking from the bottom up, was: Large, Medium and Small crystals. Details for the generation of Small, Medium and Large crystals can be found in **Sections 2.3.1.3.1, 2.3.1.3.2 and 2.3.1.3.3.** respectively. PSD data for each batch can be found in **Section 3.3.4.2.**

4.3.2.1. Effects of % RH on hydrate stability of CBZ dihydrate generated from the standard method (Medium sized particles)

In this section, the hydrate stability of 'Medium' CBZ dihydrate particles in Vial 1-A (25 °C/2% RH) is compared with that of Vial 3-A (25 °C/75% RH) (**Table 4.1**), and findings from Vial 1-C (80 °C/2% RH) are compared to those for Vial 3-C (80 °C/75% RH) (**Table 4.1**).

Tracking of changes for these samples was completed using TGA analysis. This technique offered information on weight loss, due to the water of hydration, from a small sample size/weight. The technique left enough sample for analysis even for a long overall period of sampling. TGA analysis at specific time points and experiment end points were carried out singularly, so standard deviation values are unavailable.

4.3.2.1.1. Effects of % RH on hydrate stability at 25 °C

Based on the findings of the initial stability experiment for CBZ dihydrate, **Section 4.3.1.1**, weekly sampling points were decided on for this work and where extensive stability was seen, sampling was carried out at Week 1, 2, 4, 8, 10 and 12. Week 12 was decided on

as the final sampling point should stability be found. TGA analysis was carried out on the bulk sample of 'Medium' CBZ dihydrate crystals prior to setting up the Cuspor System. Once set up, sampling was continued until the % weight loss by TGA reached 0%, and at this point the experiment was completed. The remaining sample was then also analysed by XRPD to determine the final solid-state form.

Table 4.2 shows the comparison of TGA % weight loss values across the sampling period for Vial 1-A and Vial 3-A. The dihydrate sample at the start had a % weight loss value of 11.56%. This was below the theoretical value of 13.22% for the dihydrate, however, small differences can be due to weight loss during the instrument's equilibration or if a true representative sample was not taken, e.g., the sample was not mixed properly or a sample was taken from the top of the container. At 2% RH, all moisture relating to the water of hydration was removed within the first week. This was a much shorter timeframe than the initial experiment discussed in **Section 4.3.1.1**. The highly controlled environment within the Cuspor vial is likely the reason for this. The initial experiment did not have an optimised setup, with both CBZ dihydrate and THEO monohydrate experiments taking place within the same closed chamber. By sharing the same environment generated by the phosphorous pentoxide, it's probable that its effects were not felt to their fullest extent. In hindsight, these experiments should have been done in separate chambers to maximise the rate of dehydration without another sample being present. The end point sample showed no weight loss feature (**Figure 4.11**), and only degradation was evident on the TGA profile. XRPD analysis (**Figure 4.12**) showed that the end point sample was much less resolved but showed several similarities to the anhydrous Form III CBZ. From the literature ^[117], the dehydration the CBZ dihydrate was found to result in disorder

which, from XRPD traces, showed as an unstable amorphous form which, when held for an extended period of time, then recrystallised into a mixture of anhydrous Form I and Form III CBZ. Using the previously published XRPD data ^[117] for comparison with **Figure 4.12**, the dehydration of CBZ dihydrate was confirmed by the loss of the peak at $\sim 8.95^\circ 2\theta$. Many peaks of the anhydrous Form III CBZ were present and compared well with the anhydrous Form III starting material's XRPD trace. However, the peaks of the anhydrous Form I CBZ ($9.40^\circ 2\theta$ and $19.92^\circ 2\theta$) were more difficult to pin-point due to the lower crystallinity of the solid. This lower crystallinity has previously been seen ^[117] and was attributed to a lower dehydration temperature (40-80 °C). This previously reported study then detailed how dehydration at a higher temperature (65-100 °C), once the solid recrystallised, gave an XRPD trace with better resolution than those at lower temperatures. The presence of a small peak at $\sim 12^\circ 2\theta$, which corresponds to a peak in the dihydrate trace was unexpected as all other analyses suggest the dihydrate is not present.

Table 4.2: TGA % weight loss values for 'Medium' sized CBZ dihydrate crystals at different % RH levels at 25 °C.

Vial #	Start	Wk 1	Wk 2	Wk 4	Wk 8	Wk 10	Wk 12
<i>% Weight loss (water of hydration)</i>							
1-A (2% RH, 25 °C)	11.56	0.00					
3-A (75% RH, 25 °C)	11.56	12.14	13.20	12.54	13.38	13.03	12.78

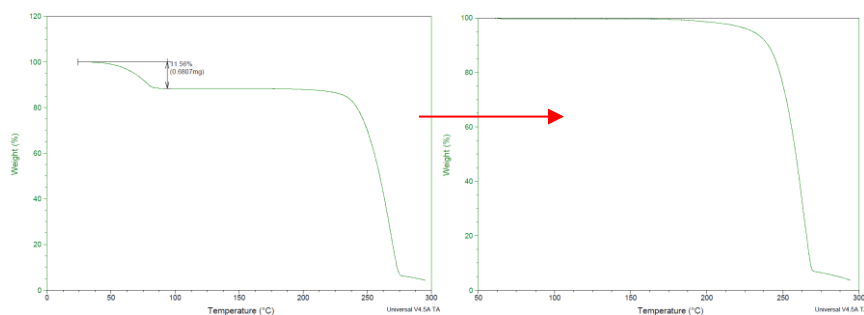


Figure 4.11: TGA analyses for 'Medium' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-A (2% RH, 25 °C).

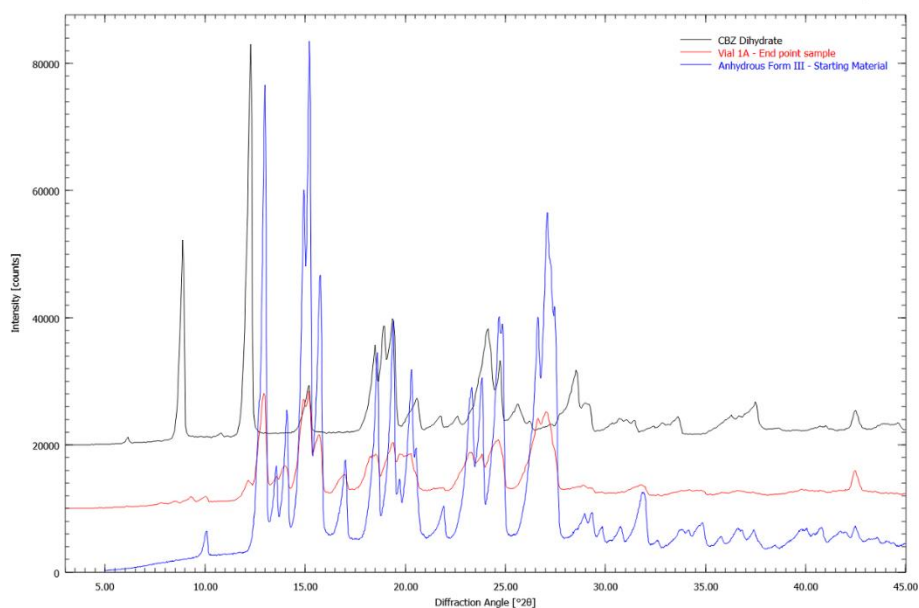


Figure 4.12: XRPD overlay for 'Medium' CBZ dihydrate crystals showing the CBZ dihydrate (black), end point sample (red) for Vial 1-A (2% RH, 25 °C) and anhydrous Form III CBZ (blue).

From **Table 4.2**, when held at 75% RH and 25 °C, stability in the % weight loss was seen. From the start to Week 12, values remained close to the 13.22% theoretical % weight loss for the water of hydration. After the experiment began, all % weight loss values were larger than the starting value, suggesting that the sample took on small amounts of moisture from the environment to fully fill its channels. Differences in values after the initial TGA value are likely to be due to instrument equilibrium, as sampling from the Cuspor System were methodically carried out to ensure proper mixing

prior to sampling. To confirm that the consistent step-like feature in the TGA results (**Figure 4.13**) seen over the 12-week period was related to the dihydrate of CBZ, XRPD analysis was carried out. **Figure 4.14** shows the end point sample to overlay well with the CBZ dihydrate from the start of the study. No similarities were found between the end point sample and that of the anhydrous Form III CBZ from which the dihydrate was originally prepared.

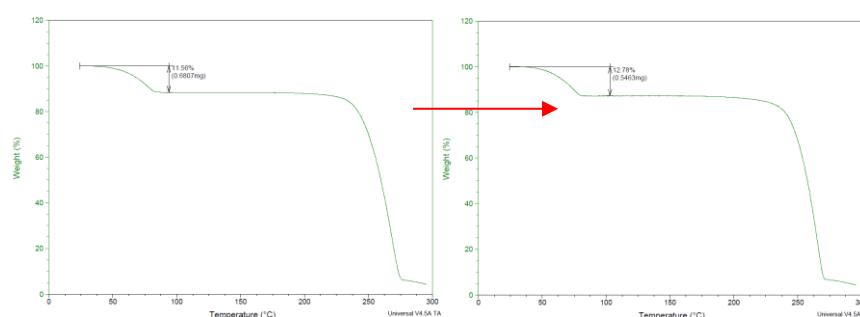


Figure 4.13: TGA analyses for 'Medium' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-A (75% RH, 25 °C).

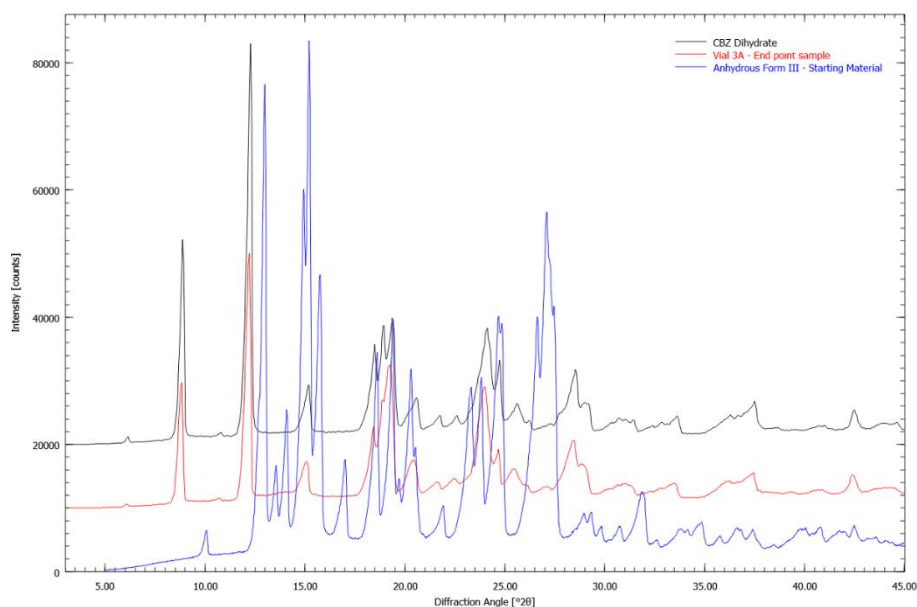


Figure 4.14: XRPD overlay for 'Medium' CBZ dihydrate crystals showing the CBZ dihydrate (black), end point sample (red) for Vial 3-A (75% RH, 25 °C) and the anhydrous Form III CBZ (blue).

At 25 °C, using 2% RH resulted in the loss of the water of hydration for 'Medium' CBZ dihydrate crystals within a week. However, in an environment of 75% RH, hydrate stability was seen up to 12 weeks.

4.3.2.1.2. Effects of % RH on hydrate stability at 80 °C

Based on the results from **Section 4.3.2.1.1** where all moisture was lost for the 2% RH sample at the first sampling time point, it was decided, for the high temperature samples, to reduce the sampling period to daily, in the hope of gathering more data on the dehydration of the sample.

Table 4.3 shows the comparison of TGA % weight loss values for Vial 1-C and Vial 3-C. With the same initial % weight loss value of 11.56% as previously mentioned, results show that regardless of which % RH was used, dehydration occurred at 80 °C within one day.

At 2% RH (Vial 1-C), the end point sample showed only evidence of degradation in the TGA (**Figure 4.15**), and XRPD analysis (**Figure 4.16**) showed that the end point sample compared well with the anhydrous Form III CBZ. No peaks characteristic of the original dihydrate were seen. While a mixture of anhydrous Form I and Form III CBZ is often seen post dehydration ^[117], with the lower crystallinity of the sample, peaks of the anhydrous Form I CBZ do not appear to be present. In particular the peak at 9.40° 2θ, while only showing as a minor peak in **Figure 4.12**, its absence in **Figure 4.16** is clear in comparison, further suggesting that only anhydrous Form III CBZ was present in the sample held at 80 °C. The higher temperature may be the reason for this, as it was previously detailed to increase the rate and extent of dehydration in comparison to lower temperatures. ^[117]

Table 4.3: TGA % weight loss values for 'Medium' sized CBZ dihydrate crystals at different % RH levels at 80 °C.

Vial #	Start	Day 1
<i>% Weight loss (water of hydration)</i>		
1-C (2% RH, 80 °C)	11.56	0.00
3-C (75% RH, 80 °C)	11.56	0.00

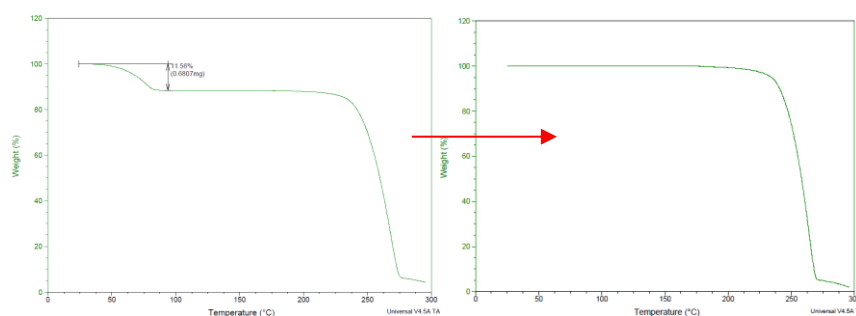


Figure 4.15: TGA analyses for 'Medium' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-C (2% RH, 80 °C).

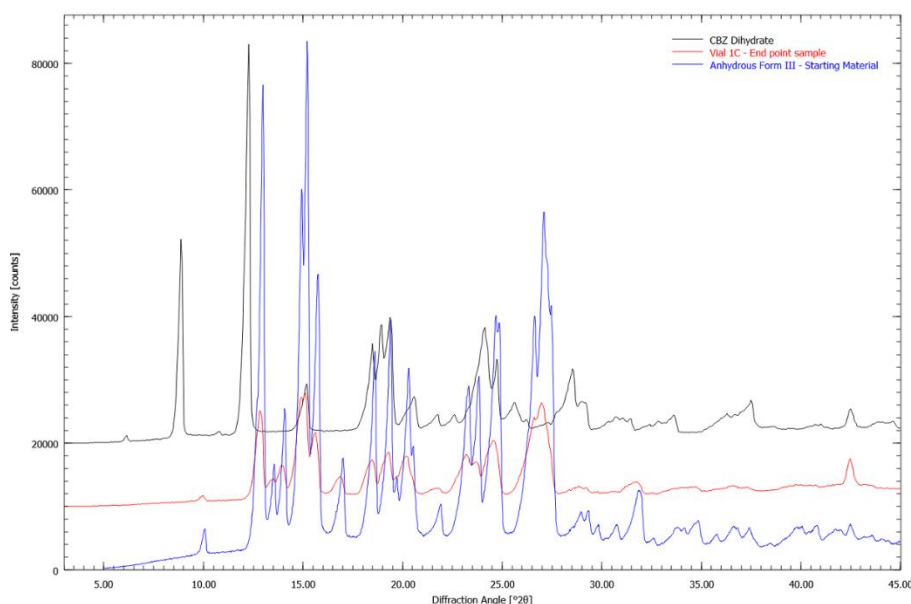


Figure 4.16: XRPD overlay for ‘Medium’ CBZ dihydrate crystals showing the CBZ dihydrate (black), end point sample (red) for Vial 1-C (2% RH, 80 °C) and anhydrous CBZ, Form III (blue).

The end point sample of Vial 3-C showed similar results to that of Vial 1-C, with degradation the only feature on the TGA graph (**Figure 4.17**) and the XRPD diffractogram showing the sample to once again compare well with the anhydrous Form III CBZ (**Figure 4.18**), with no peaks characteristic of the dihydrate form observed. Similar to what was found for Vial 1-C (and unlike Vial 3-A, 75%RH/25 °C), peaks of the anhydrous Form I CBZ do not appear to be present and the higher temperature (compared to Vial 3-A) may be the reason for this. ^[117] At both levels of % RH and 80 °C, CBZ dihydrate appears to have transitioned back to the original, Form III CBZ anhydrous starting material within one day. Hydrate stability at 80 °C was not evident.

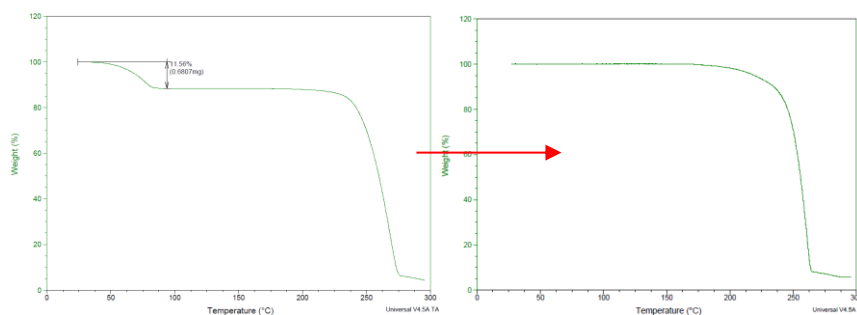


Figure 4.17: TGA analyses for 'Medium' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-C (75% RH, 80 °C).

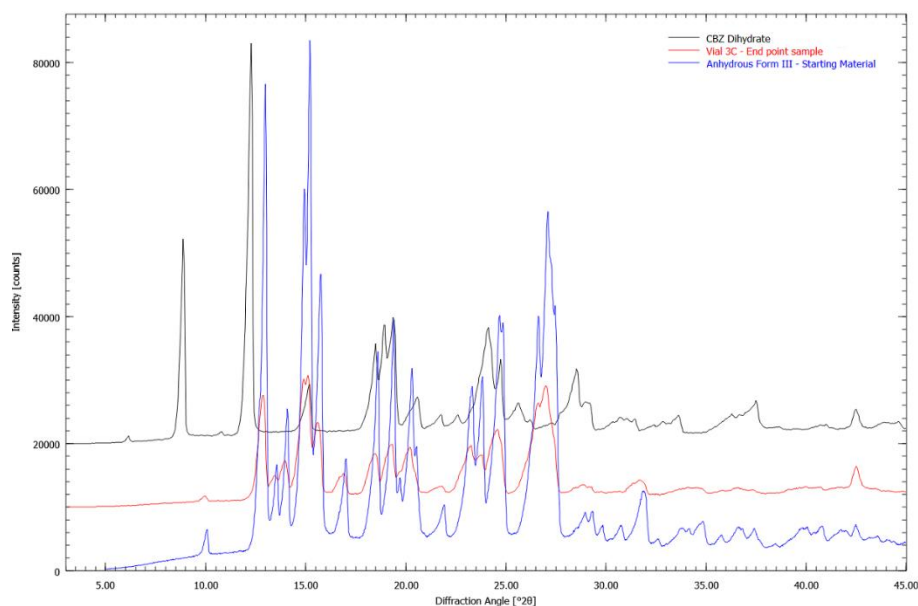


Figure 4.18: XRPD overlay for 'Medium' CBZ dihydrate crystals showing the CBZ dihydrate (black), end point sample (red) for Vial 3-C (75% RH, 80 °C) and the anhydrous Form III CBZ (blue).

4.3.2.1.3. Hydrate stability at centre point conditions:40% RH/50 °C

Based on the conditions available, using the Cuspor Ageing System, the dehydration of CBZ dihydrate under centre point conditions was also examined. This sample was set up at the same time as Vial 1-A and 3-A discussed in **Section 4.3.2.1.1**; therefore, weekly sampling points were decided on when starting this experiment.

Table 4.4 shows that the dihydrate lost all water of hydration within the first week at 40% RH and 50 °C. Daily sampling would have been more informative for these conditions. TGA results for this sample (**Figure 4.19**) showed only degradation, as was seen for the end point sample of Vials 1-A (**Figure 4.11**), 1-C (**Figure 4.15**) and 3-C (**Figure 4.17**). XRPD analysis of the end point sample of Vial 2-B is shown in **Figure 4.20** and showed a trace which compared very well to the anhydrous Form III CBZ. Thus, hydrate stability was not found at 40% RH and 50 °C.

Table 4.4: TGA % weight loss values for ‘Medium’ sized CBZ dihydrate crystals at 40% RH and 50 °C.

Vial #	Start	Wk 1
<i>% Weight loss (water of hydration)</i>		
2-B (40% RH, 50 °C)	11.56	0.00

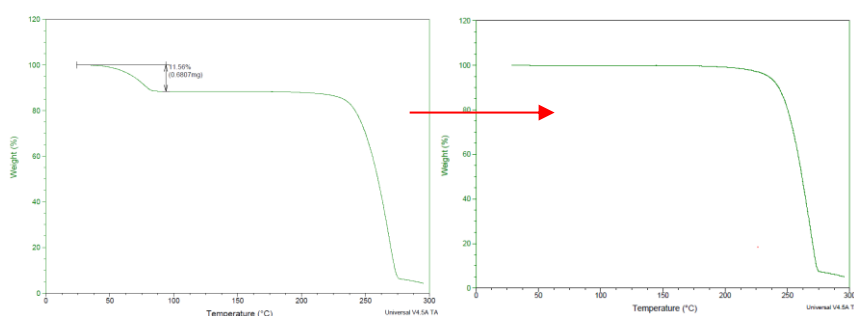


Figure 4.19: TGA analyses for ‘Medium’ CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 2-B (40% RH, 50 °C).

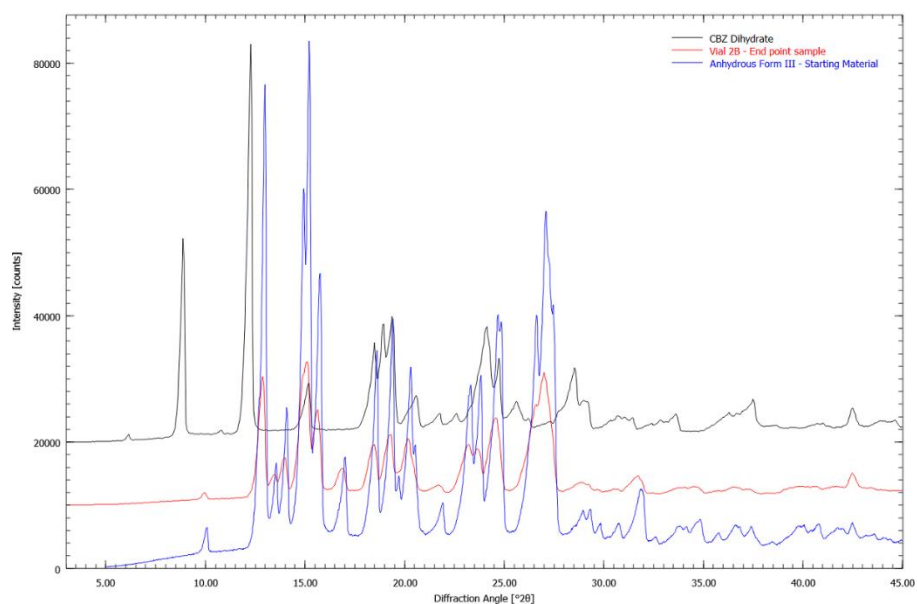


Figure 4.20: XRPD overlay for ‘Medium’ CBZ dihydrate crystals showing the CBZ dihydrate (black), end point sample (red) for Vial 2-B (40% RH, 50 °C) and anhydrous Form III CBZ (blue).

4.3.2.2. Effects of temperature on hydrate stability of CBZ dihydrate generated from the standard method (Medium sized particles)

In this section, the effects of temperature on hydrate stability ‘Medium’ CBZ dihydrate particles are examined, comparing:

- Vial 1-A (25 °C/2% RH) vs. Vial 1-C (80 °C/2% RH) (**Table 4.1**)

Vial 3-A (25 °C/2% RH) vs. Vial 3-C (80 °C/2% RH) (**Table 4.1**) **Table 4.5** shows the comparison of TGA % weight loss values across the sampling periods for Vial 1-A and Vial 1-C (2% RH). Both samples reached 0.00% weight loss at their first respective sampling points. Due to differences in sampling points, comparing these samples is somewhat difficult. However, as Vial 1-C had reached a weight loss value of 0.00% after only one day, it can be deduced that this would also be the case after one week. TGA graphs of both showed only

degradation (**Figure 4.11** and **Figure 4.15**) and, as previously indicated, XRPD analysis (**Figure 4.12** and **Figure 4.16**) showed that both end point samples had XRPD traces that compared well to that of the anhydrous Form III CBZ.

Table 4.5: TGA % weight loss values for ‘Medium’ sized CBZ dihydrate crystals at different temperatures at 2% RH.

Vial #	Start	Wk 1
<i>% Weight loss (water of hydration)</i>		
1-A (2% RH, 25 °C)	11.56	0.00
	Start	Day 1
1-C (2% RH, 80 °C)	11.56	0.00

Regardless of temperature, 2% RH was too low to provide an environment which would offer stability for ‘Medium’ CBZ dihydrate crystals. Under both sets of conditions the hydrated form was lost.

Table 4.6 shows the comparison of TGA % weight loss values across the sampling periods for Vial 3-A and Vial 3-C (75% RH). Hydrate stability was seen at the lower temperature of 25 °C and up to a 12-week period. However, at 80 °C hydrate stability was lost within the first day, suggesting the higher temperature drove the dehydration of the sample under those conditions. TGA results (**Figure 4.13** and **Figure 4.17**) and XRPD analysis (**Figure 4.14** and **Figure 4.18**) confirmed the presence of the dihydrate in Vial 3-A while Vial 3-C had an end point sample which is believed to be the anhydrous Form III CBZ.

Table 4.6: TGA % weight loss values for ‘Medium’ sized CBZ dihydrate crystals at different temperatures at 75% RH.

Vial #	Start	Wk 12
% Weight loss (water of hydration)		
3-A (75% RH, 25 °C)	11.56	12.78
	Start	Day 1
3-C (75% RH, 80 °C)	11.56	0.00

This is the first glimpse into a hydrate stability design space which could be used to prepare a future drying study plan that is data driven and is coming from a place of knowledge.

4.3.2.3. Overall hydrate stability dependence on humidity and temperature for CBZ dihydrate generated from the standard method (Medium sized particles)

Figure 4.21 shows an overlay of all end point samples along with the initial dihydrate trace and the Form III CBZ anhydrous starting material. Vial 3-A was the only sample to have a trace which overlaid with the dihydrate pattern, meaning the conditions of 75% RH and 25 °C allowed for hydrate stability over the 12-week period for CBZ dihydrate. All other XRPD traces of end point samples (Vial 1-A, 2-B, 1-C and 3-C) compared well with the Form III CBZ anhydrous starting material. The only other difference noted was for Vial 1-A where a peak at 9.40° 2θ was found, and this peak is characteristic of the anhydrous Form I CBZ.

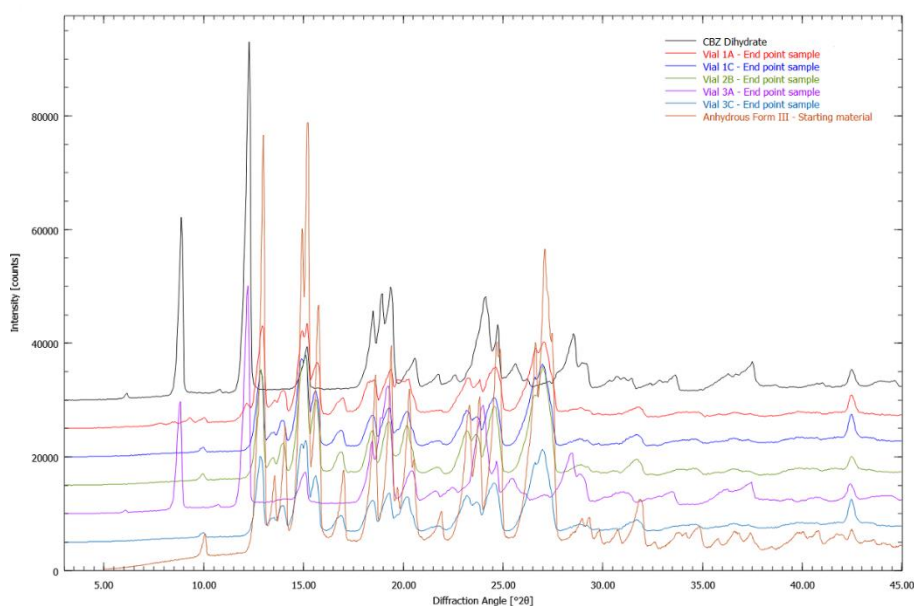


Figure 4.21: XRPD overlay for 'Medium' CBZ dihydrate crystals showing the CBZ dihydrate (black), end point samples of Vial 1-A (red), Vial 1-C (dark blue), Vial 2-B (green), Vial 3-A (purple), Vial 3-C (light blue) and the anhydrous Form III CBZ (dark orange).

Figure 4.22 provides a summary of the hydrate stability study results showing the form of the end point samples recovered. This will be an important tool when devising drying study experiments to determine conditions where the dry hydrate form can be obtained.

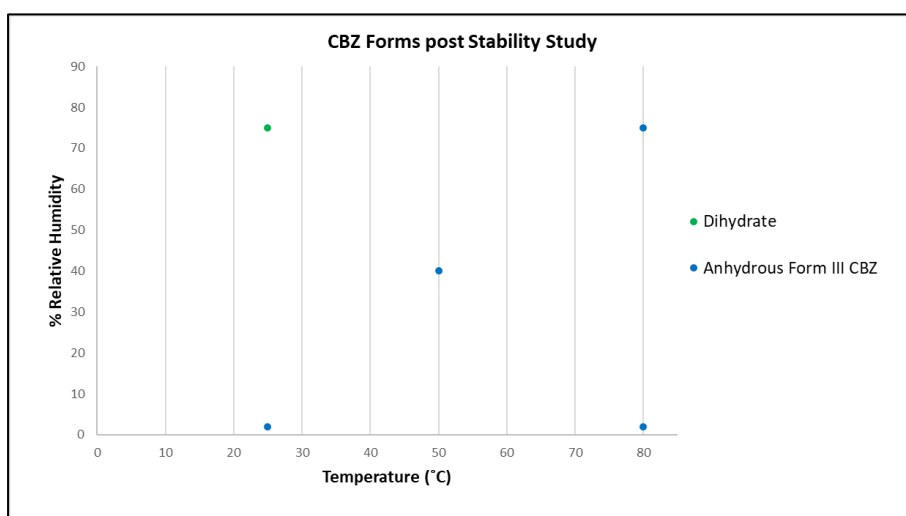


Figure 4.22: Solid-state form diagram for CBZ end point samples, post Stability Study using the Cuspor Ageing System.

4.3.2.4. Effects of PSD on hydrate stability of CBZ dihydrate

As discussed at the beginning of **Section 4.3.2**, each vial contained portions of crystals with differing PSD. By comparing the TGA % weight loss values for each sample within each vial, the effects of PSD at those specific conditions can be analysed.

4.3.2.4.1. Effects of PSD on hydrate stability at 2% RH, 25 °C

Hydrate stability was lost for each of the samples, regardless of PSD, when held at 2% RH and 25 °C for one week (**Table 4.7**). Differences in available surface area did not impact the level of dehydration within the sampling period. TGA analyses of 'Small', 'Medium' and 'Large' crystals showed only degradation for each end point sample (see **Figure 4.23**, **Figure 4.11** and **Figure 4.24** respectively). From XRPD analysis (**Figure 4.25**), each sample compared well with the anhydrous Form III CBZ. Resolution of end point samples was reduced but the presence of a small peak at 9.40° 2θ, in each of the samples, relates to a peak in the anhydrous Form I CBZ. ^[117] Another common feature, was the peak at ~12° 2θ, which was a peak found in the diffractogram of the dihydrate, suggesting the end point samples were a mixture of forms. A mixture of both anhydrous forms, post dehydration has been seen previously ^[117] but the dihydrate peak was unusual as all other analysis suggests no dihydrate was present.

Table 4.7: TGA % weight loss values for CBZ dihydrate crystals of differing PSD at 2% RH and 25 °C (Vial 1-A).

Vial 1-A	Start	Wk 1
% Weight loss (water of hydration)		
Small	13.17	0.00
Medium	11.56	0.00
Large	10.94	0.00

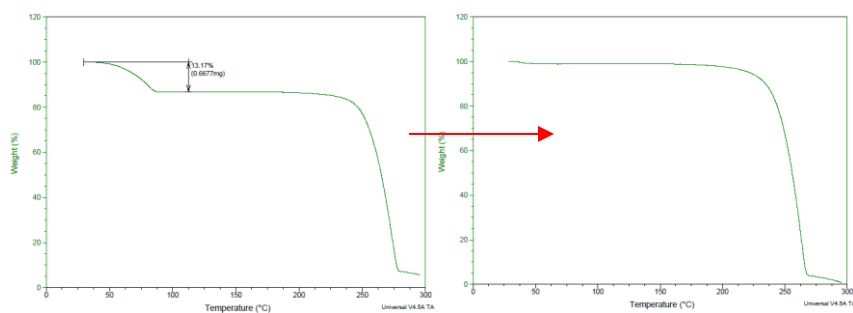


Figure 4.23: TGA analyses for 'Small' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-A (2% RH, 25 °C).

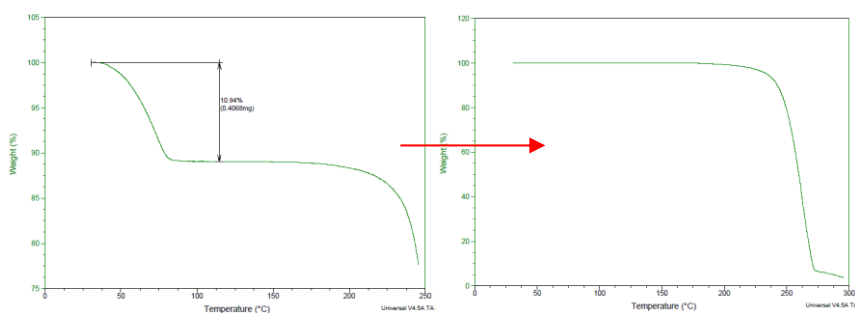


Figure 4.24: TGA analyses for 'Large' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-A (2% RH, 25 °C).

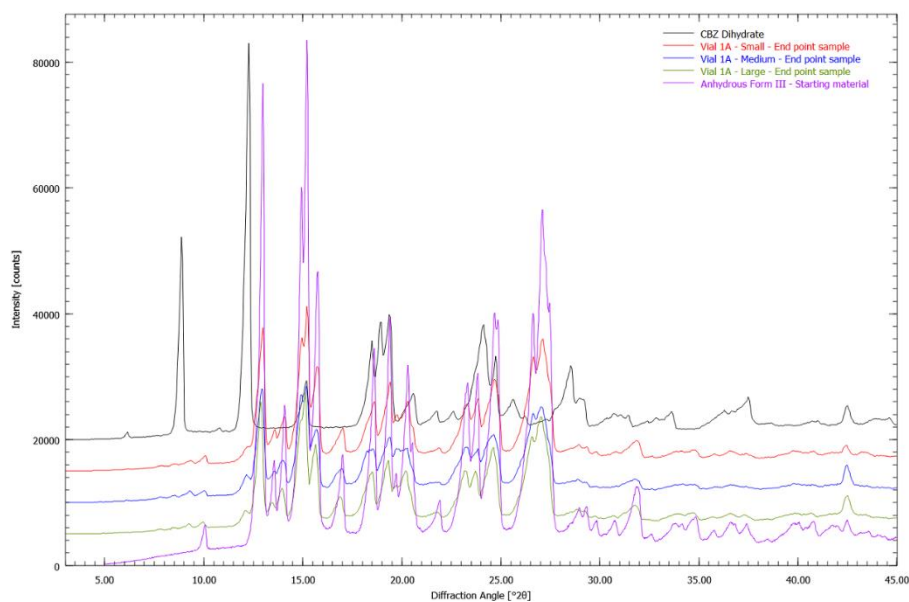


Figure 4.25: XRPD overlay for CBZ dihydrate (black), end point samples of 'Small' (red), 'Medium' (dark blue), 'Large' (green) CBZ crystals from Vial 1-A and anhydrous Form III CBZ (purple).

Changing PSD of CBZ dihydrate crystals does not provide protection against dehydration of the dihydrate when held at 2% RH and 25 °C.

4.3.2.4.2. Effects of PSD on hydrate stability at 75% RH, 25 °C

From **Table 4.8**, results showed that hydrate stability across each of the samples was maintained when held at 75% RH and 25 °C for up to a 12-week period. Differences in TGA % weight loss values from the theoretical value of ~13% was likely due to the instrument equilibrating prior to the analysis beginning. Sampling from the Cuspor Ageing System was carried out in the same way at each sampling point, ensuring proper mixing to obtain a representative sample. TGA analyses of 'Small', 'Medium' and 'Large' crystals all showed the step-like feature with a % weight loss close to the expected value for each end point sample, (see **Figure 4.26**, **Figure 4.13** and **Figure 4.27** respectively). From XRPD analysis (**Figure 4.28**), each sample contained peaks characteristic of the dihydrate form. However, the traces for 'Small' and 'Large' crystals also showed peaks relating to the anhydrous starting material, possibly due to the beginning of a transition back to the original anhydrous starting material, and ultimately the start of the dehydration of the dihydrate form. The 'Medium' crystals, generated by the standard procedure (**Section 2.3.1.2**) contained only the peaks of the dihydrate form.

This set of conditions provided the first indications that differences in PSD can affect the dehydration of CBZ dihydrate over a 12-week period, however it is unclear why 'Medium' crystals should differ from 'Large' or 'Small' crystals.

Table 4.8: TGA % weight loss values for CBZ dihydrate crystals of increasing PSD at 75% RH and 25 °C (Vial 3-A).

Vial 3-A	Start	Wk 1	Wk 2	Wk 4	Wk 8	Wk 10	Wk 12
<i>% Weight loss (water of hydration)</i>							
Small	13.17	13.38	13.04	12.59	13.10	12.98	13.08
Medium	11.56	12.14	13.20	12.54	13.38	13.00	12.78
Large	10.94	10.02	11.47	13.01	11.08	11.86	10.77

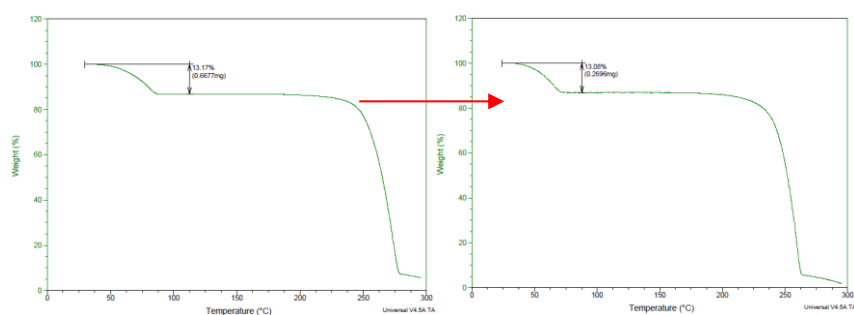


Figure 4.26: TGA analyses for 'Small' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-A (75% RH, 25 °C).

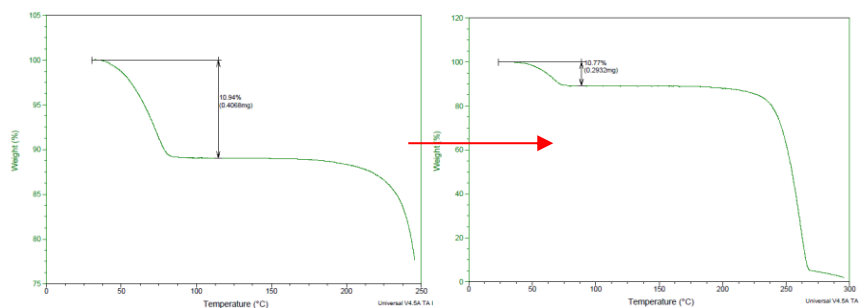


Figure 4.27: TGA analyses for 'Large' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-A (75% RH, 25 °C).

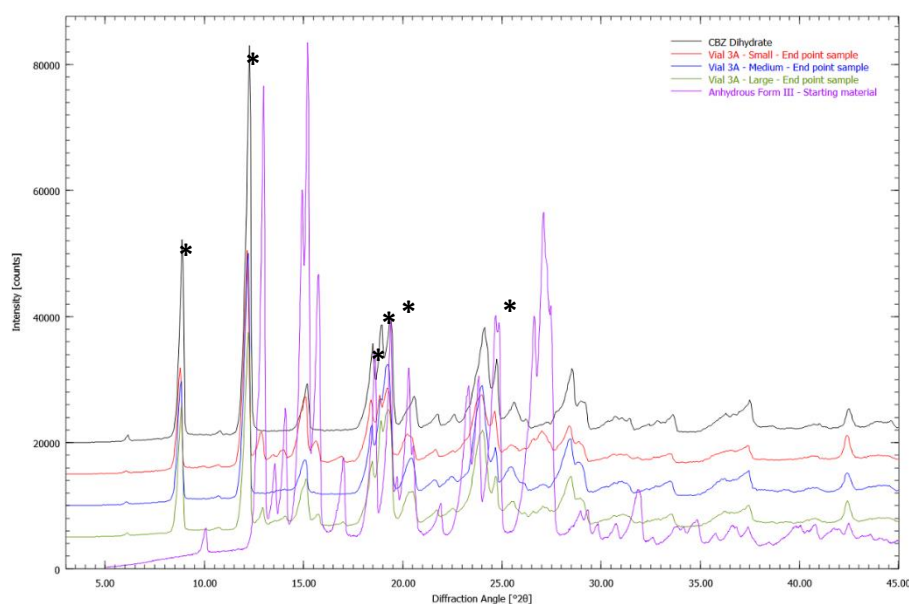


Figure 4.28: XRPD overlay for CBZ dihydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) CBZ crystals from Vial 3-A and anhydrous Form III CBZ (purple). * denotes the characteristic peaks of the dihydrate.

4.3.2.4.3. Effects of PSD on hydrate stability at 40% RH, 50 °C

Results showed that hydrate stability at 40% RH and 50 °C, for ‘Small’ and ‘Medium’ crystals was lost within one week, whereas ‘Large’ crystals lost all water of hydration within two weeks (**Table 4.9**). TGA analyses of ‘Small’ and ‘Medium’ samples showed only degradation after one week at these conditions, whereas for ‘Large’ crystals, there was a small weight loss at the one-week sampling point. At the next sampling point, only degradation was seen (see **Figure 4.29**, **Figure 4.19** and **Figure 4.30** respectively). XRPD analysis (**Figure 4.31**) of all end point samples compared well with the anhydrous Form III CBZ. There was no common peak with the dihydrate form observed, as was seen in **Section 4.3.2.4.1**.

Table 4.9: TGA % weight loss values for CBZ dihydrate crystals of increasing PSD at 40% RH and 50 °C (Vial 2-B).

Vial 2-B	Start	Wk 1	Wk 2
<i>% Weight loss (water of hydration)</i>			
Small	13.17	0.00	
Medium	11.56	0.00	
Large	10.94	0.93	0.00

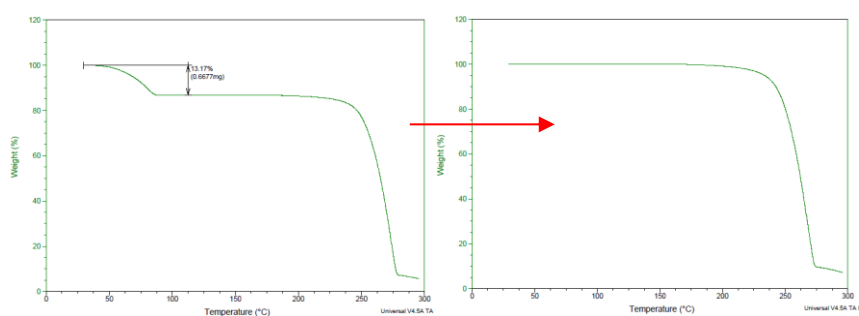


Figure 4.29: TGA analyses for 'Small' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 2-B (40% RH, 50 °C).

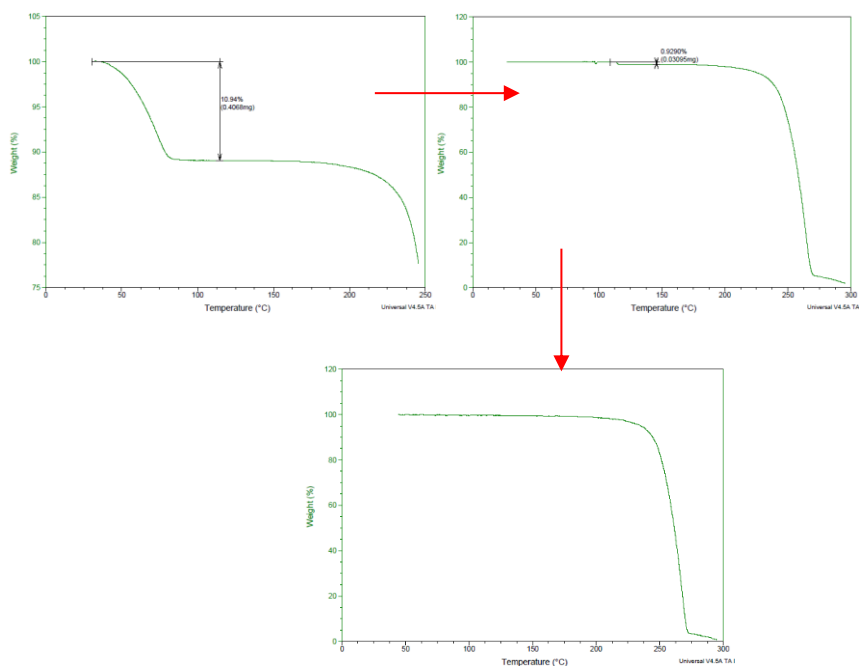


Figure 4.30: TGA analyses for 'Large' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 2B (40% RH, 50 °C).

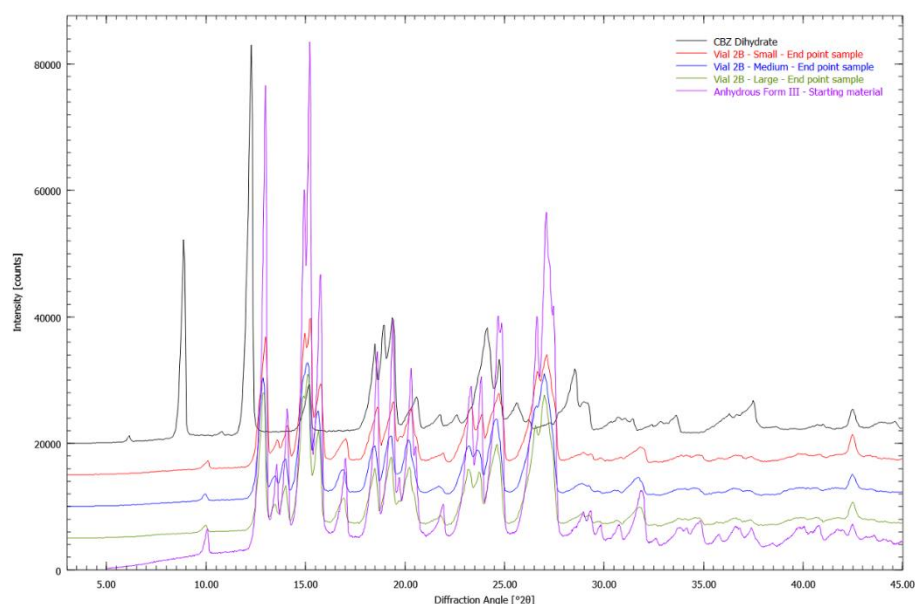


Figure 4.31: XRPD overlay for CBZ dihydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) CBZ crystals from Vial 2-B and anhydrous Form III CBZ starting material (purple).

Despite the TGA % weight loss values of ‘Large’ dihydrate crystals being lower than the other two samples initially, it took longer for full dehydration to occur. This may be related to moisture having to move through the longer channels of the larger crystals. Thus, at these conditions, the stability of the dihydrate form is impacted by PSD differences.

4.3.2.4.4. Effects of PSD on hydrate stability at 2% RH, 80 °C

Results showed that hydrate stability across each of the samples was lost when held at 2% RH and 80 °C for one day (**Table 4.10**). Differences in PSD did not impact the level of dehydration within the sampling period. TGA analyses of ‘Small’, ‘Medium’ and ‘Large’ crystals showed only degradation for each end point sample (see **Figure 4.32**, **Figure 4.15** and **Figure 4.33** respectively). From XRPD analysis (**Figure 4.34**), end points of each sample contained peaks relating to the anhydrous Form III CBZ. There was no common peak with the dihydrate form observed, as was seen in **Section 4.3.2.4.1**.

Table 4.10: TGA % weight loss values for CBZ dihydrate crystals of increasing PSD at 2% RH and 80 °C (Vial 1-C).

Vial 1-C	Start	Day 1
% Weight loss (water of hydration)		
Small	13.17	0.00
Medium	11.56	0.00
Large	10.94	0.00

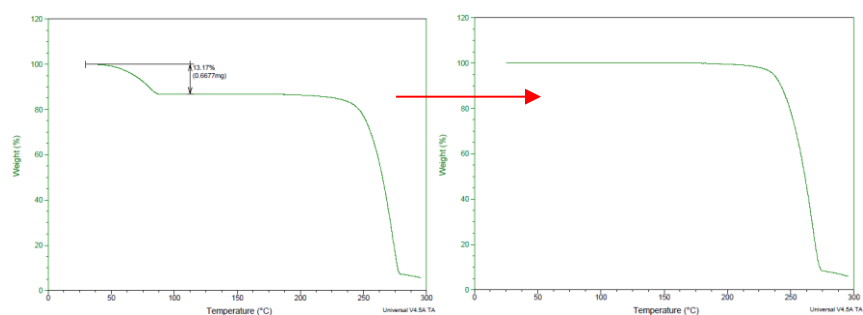


Figure 4.32: TGA analyses for 'Small' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-C (2% RH, 80 °C).

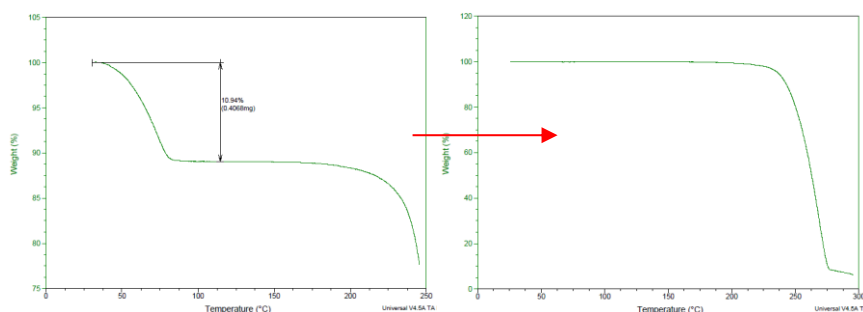


Figure 4.33: TGA analyses for 'Large' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 1-C (2% RH, 80 °C).

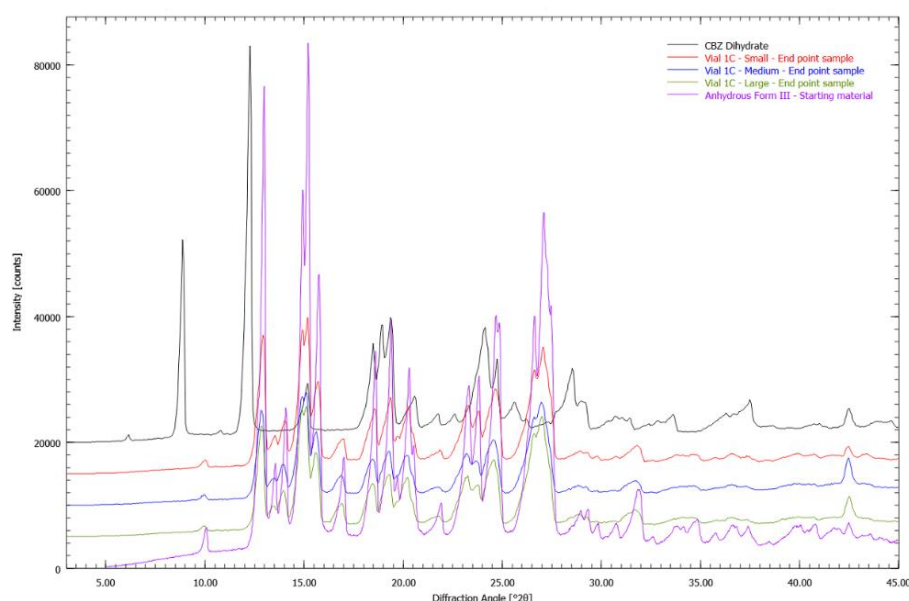


Figure 4.34: XRPD overlay for CBZ dihydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) CBZ crystals from Vial 1-C and anhydrous Form III CBZ (purple).

At 2% RH and 80 °C, hydrate stability was not seen at the day 1 timepoint for all CBZ sample, irrespective of PSD.

4.3.2.4.5. Effects of PSD on hydrate stability at 75% RH, 80 °C

Results showed that hydrate stability for each sample was lost when held at 75% RH and 80 °C for one week (**Table 4.11**). Differences in PSD did not impact the level of dehydration within the sampling period. TGA analyses of ‘Small’, ‘Medium’ and ‘Large’ crystals showed only degradation for each end point sample (see **Figure 4.35**, **Figure 4.17** and **Figure 4.36** respectively). XRPD analysis of the same samples (**Figure 4.37**) showed each end point sample compared well with the anhydrous Form III CBZ, with no peaks of the dihydrate form observed, as was seen in **Section 4.3.2.4.1**.

Table 4.11: TGA % weight loss values for CBZ dihydrate crystals of increasing PSD at 75% RH and 80 °C (Vial 3-C).

Vial 3-C	Start	Day 1
<i>% Weight loss (water of hydration)</i>		
Small	13.17	0.00
Medium	11.56	0.00
Large	10.94	0.00

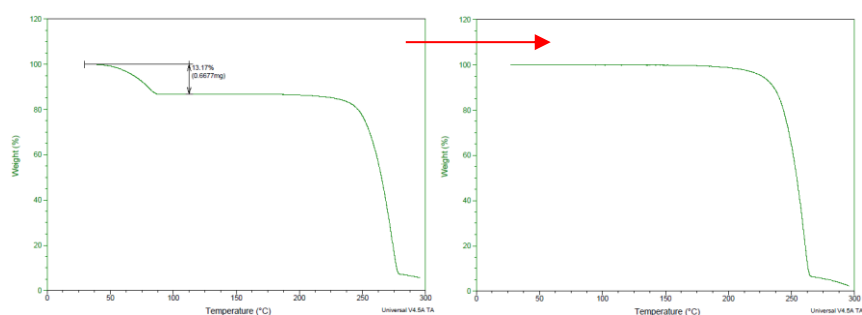


Figure 4.35: TGA analyses for 'Small' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-C (75% RH, 80 °C).

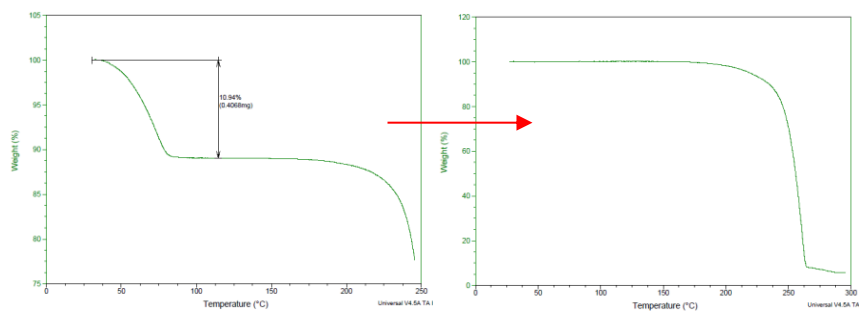


Figure 4.36: TGA analyses for 'Large' CBZ dihydrate crystals showing the start (left) and end (right) point samples for Vial 3-C (75% RH, 80 °C).

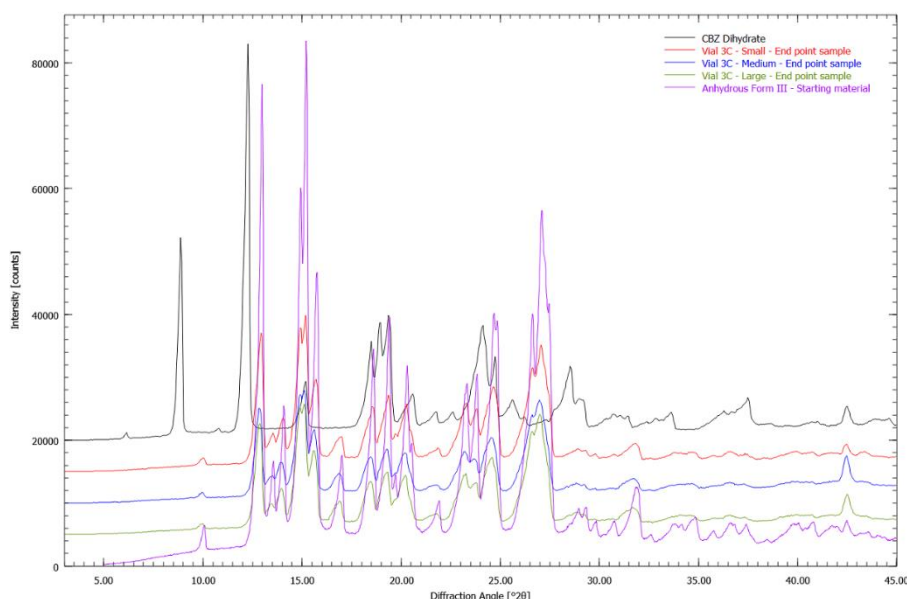


Figure 4.37: XRPD overlay for CBZ dihydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) CBZ crystals from Vial 3-C and anhydrous Form III CBZ (purple).

Similar to the previous section, at 75% RH and 80 °C, no hydrate stability was found after one day for all CBZ samples, irrespective of PSD.

4.3.3. Hydrate Stability Study – THEO Monohydrate

Like **Section 4.3.2**, the stability study of THEO monohydrate sample was carried out in the Cuspor Ageing System (**Figure 2.4**). The conditions chosen were also the same cross section used for CBZ dihydrate samples. **Table 4.12** below shows this cross section.

Table 4.12: Hydrate stability study conditions for THEO Monohydrate.

THEO Monohydrate			
	25 °C	50 °C	80 °C
2 %RH	Vial 4-A		Vial 4-C
40 %RH		Vial 5-B	
75 %RH	Vial 6-A		Vial 6-C

As for CBZ, each large outer vial contained three samples of THEO monohydrate: Small, Medium and Large crystals. For each vial, the stacking from the bottom up, went: Large, Medium and Small crystals. In **Section 4.3.3.1** and **Section 4.3.3.2**, results discussed are of the 'Medium' sized THEO monohydrate particles. Details for the generation of Large, Medium and Small crystals can be found in **Section 2.3.1.7.1**, **2.3.1.7.2** and **2.3.1.7.3**. PSD data for each batch can be found in **Section 3.3.4.3**.

4.3.3.1. Effects of % RH on hydrate stability of THEO monohydrate generated from the standard method (Medium sized particles)

In this section, the hydrate stability of 'Medium' THEO monohydrate particles in Vial 4-A (25 °C/2% RH) is compared with that of Vial 6-A (25 °C/75% RH) (**Table 4.13**), and findings from Vial 4-C (80 °C/2% RH) are compared with Vial 6-C (80 °C/75% RH) (**Table 4.14**). TGA analysis was again used to track changes in each sample at each sampling period. TGA analysis at specific time points and experiment end points were carried out singularly so standard deviation values are unavailable.

4.3.3.1.1. Effects of % RH on hydrate stability at 25 °C

Based on the results of the initial stability experiment for THEO monohydrate (**Section 4.3.1.2**), weekly sampling points were chosen for 25 °C samples and where longer-term stability was seen, sampling was carried out at Week 1, 2, 4, 8 and 12 time points. In comparison to CBZ dihydrate work, the Week 10 sampling point was missed. Week 12 was decided on as the final sampling point. TGA analysis was carried out on the bulk sample of 'Medium' CBZ dihydrate crystals prior to setting up the Cuspor System. Once set up, sampling was continued until the % weight loss reached 0%,

which denoted the end of the experiment. XRPD analysis was carried out on the remaining sample to determine the final solid-state form.

Table 4.13 shows the comparison of TGA % weight loss values across the sampling period for both Vial 4-A and Vial 6-A. The monohydrate sample had a start % weight loss value of 8.34%, which is slightly below the theoretical value of 9.08%, however, as previously mentioned these small differences can be due to weight loss during the instrument's equilibration or if a true representative sample was not taken. At 2% RH, all moisture relating to the water of hydration was removed within the first week. This was less time than the initial experiment discussed in **Section 4.3.1.2**. The highly controlled environment within the Cuspor vial is likely the reason for this. As previously mentioned in **Section 4.3.2.1.1**, the initial experiment did not have an optimised setup. By sharing the same environment generated by the phosphorous pentoxide, it's possible that its effects were not felt to their fullest extent due to the two samples being present. The end point sample showed only degradation (**Figure 4.38**) on the TGA graph. XRPD analysis (**Figure 4.39**) showed that the end point sample, while less resolved, likely due to sample size, overlaid well with the anhydrous Form II THEO.

Table 4.13: TGA % weight loss values for 'Medium' sized THEO monohydrate crystals at different % RH levels at 25 °C.

Vial #	Start	Wk 1	Wk 2	Wk 4	Wk 8	Wk 10	Wk 12
<i>% Weight loss (water of hydration)</i>							
4-A (2% RH, 25 °C)	8.34	0.00					
6-A (75% RH, 25 °C)	8.34	8.83	8.82	8.88	8.45		7.74

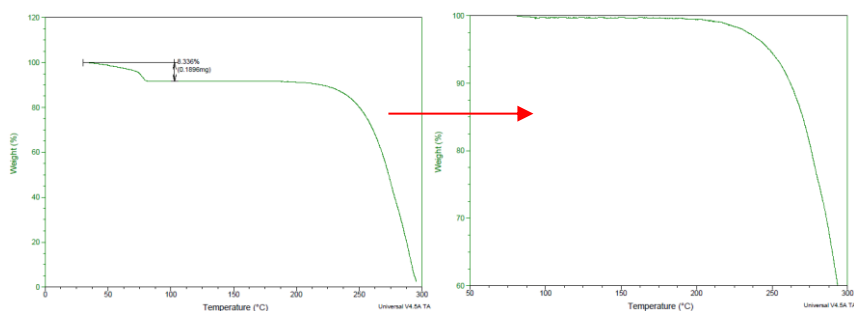


Figure 4.38: TGA analyses for 'Medium' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-A (2% RH, 25 °C).

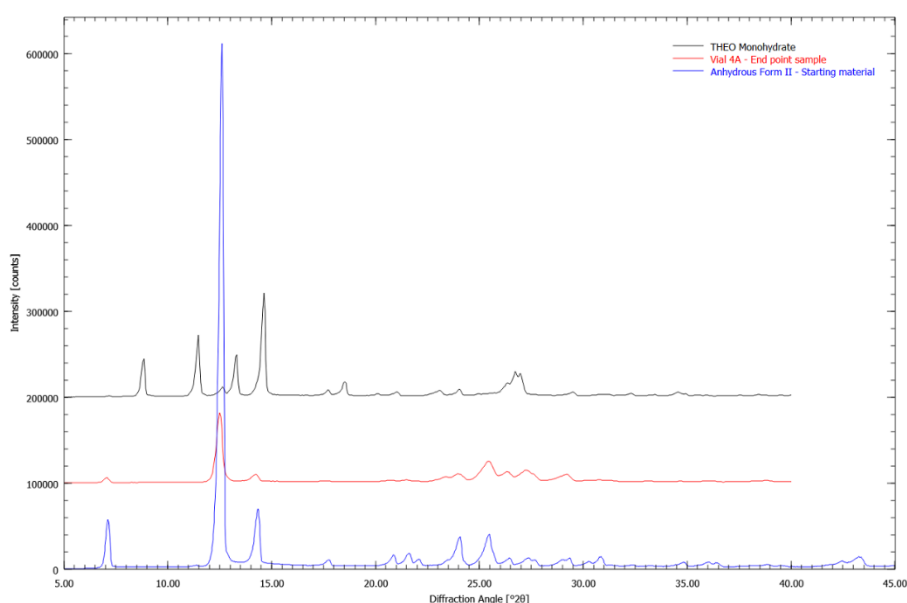


Figure 4.39: XRPD overlay for 'Medium' THEO monohydrate crystals showing the THEO monohydrate (black), end point sample (red) for Vial 1-A (2% RH, 25 °C) and the anhydrous Form II THEO (blue).

At 75% RH (**Table 4.13**) TGA values relating to the monohydrate were determined across the 12-week period. Sampling from the Cuspor vials was carried out carefully to ensure a representative sample, so differences to the theoretical value of 9.08% are likely due to equilibration of the instrument itself. To confirm that the consistent step-like feature in the TGA results (**Figure 4.40**) seen over the 12-week period was due to the monohydrate, XRPD analysis was carried out. **Figure 4.41** shows the end point sample

had a trace which closely resembled that of the THEO monohydrate. No similarities were found between the end point sample and that of the anhydrous Form II THEO.

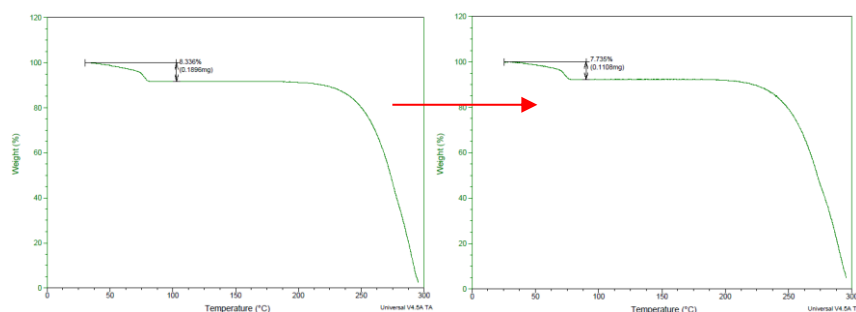


Figure 4.40: TGA analyses for 'Medium' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-A (75% RH, 25 °C).

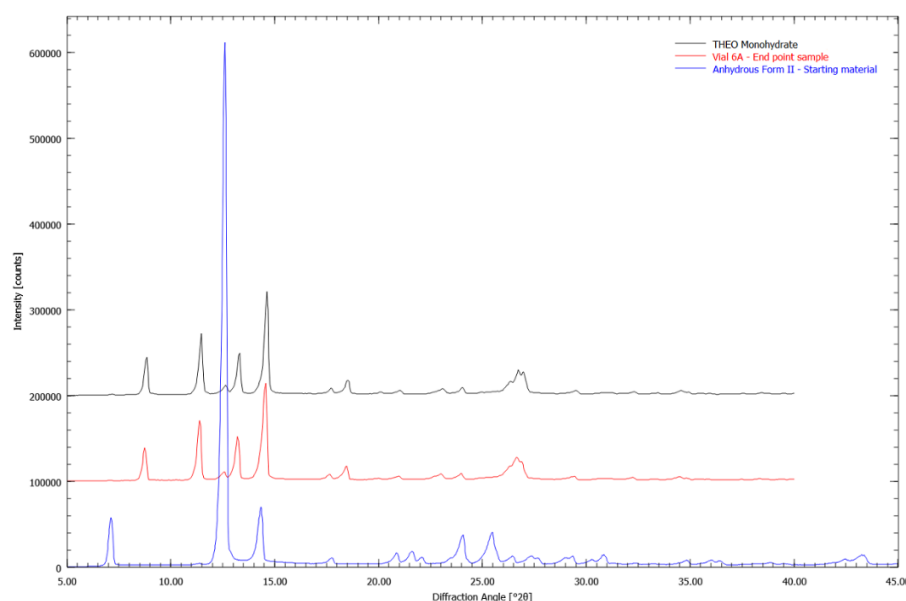


Figure 4.41: XRPD overlay for 'Medium' THEO monohydrate crystals showing the THEO monohydrate (black), end point sample (red) for Vial 6-A (75% RH, 25 °C) and anhydrous Form II THEO (blue).

At 2% RH, no hydrate stability was found after the first week and the sample was shown to have transitioned from the dihydrate back to the anhydrous starting material within the same period,

whereas at 75%, the monohydrate remained stable for the entire 12-week sampling period.

4.3.3.1.2. Effects of % RH on hydrate stability at 80 °C

Similar to **Section 4.3.2.1.2**, it was decided for the high temperature samples, to sample daily, in the hopes of gathering more dehydration data for the samples. **Table 4.14** shows the comparison of TGA % weight loss values for both Vial 4-C and Vial 6-C. With the same initial % weight loss value of 8.34% as previously mentioned, results show that regardless of either % RH value, dehydration occurred at 80 °C within one day. At 2% RH, the end point sample showed only degradation in its TGA results (**Figure 4.42**) and XRPD analysis (**Figure 4.43**) showed that the end point sample contained all the peaks of the anhydrous Form II THEO, with no peaks common to the monohydrate.

Table 4.14: TGA % weight loss values for 'Medium' sized THEO monohydrate crystals at different % RH levels at 80 °C.

Vial #	Start	Day 1
% Weight loss (water of hydration)		
4-C (2% RH, 80 °C)	8.34	0.00
6-C (75% RH, 80 °C)	8.34	0.00

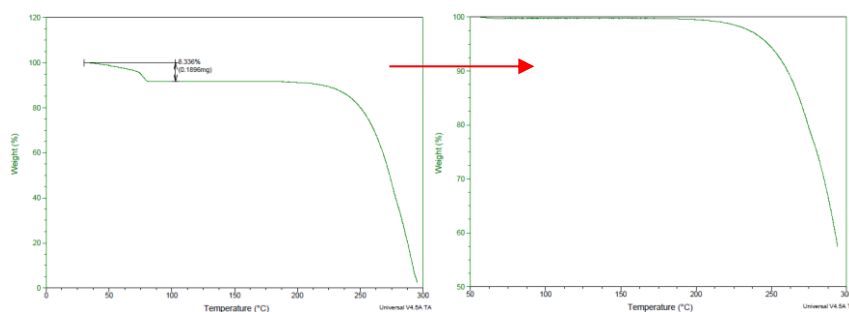


Figure 4.42: TGA analyses for 'Medium' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-C (2% RH, 80 °C).

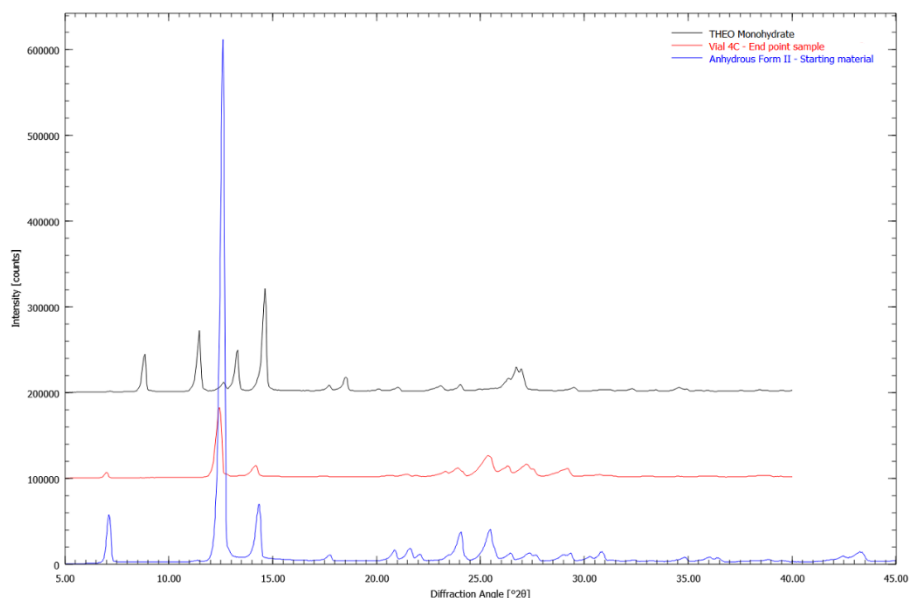


Figure 4.43: XRPD overlay for 'Medium' THEO monohydrate crystals showing the THEO monohydrate (black), end point sample (red) for Vial 4-C (2% RH, 80 °C) and anhydrous Form II THEO (blue).

The end point sample of Vial 6-C had very similar results, with degradation the only feature on its TGA graph (**Figure 4.44**), and the XRPD trace contained all the peaks of the Form II THEO anhydrous starting material (**Figure 4.45**), with no peaks common to the monohydrate form.

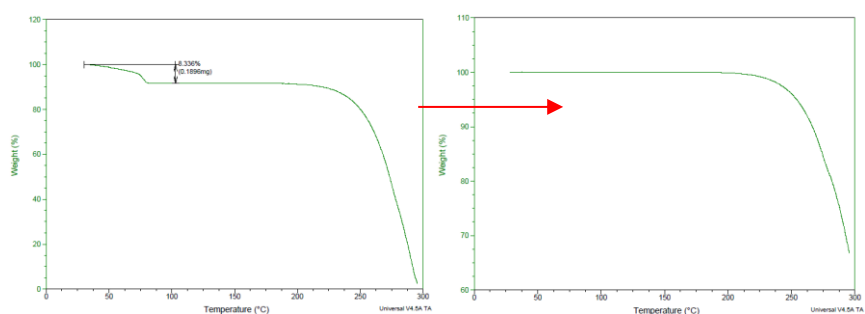


Figure 4.44: TGA analyses for 'Medium' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-C (75% RH, 80 °C).

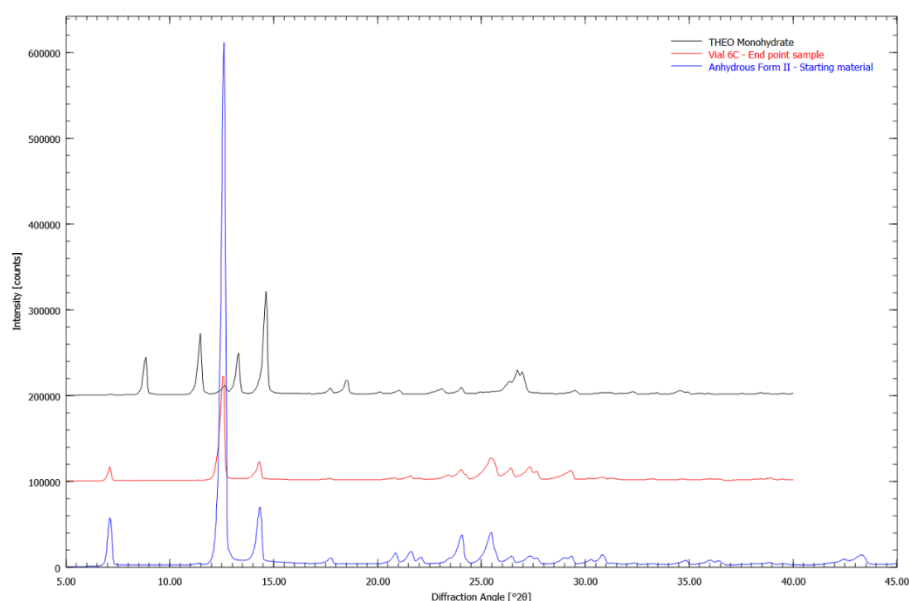


Figure 4.45: XRPD overlay for ‘Medium’ THEO monohydrate crystals showing the THEO monohydrate (black), end point sample (red) for Vial 6-C (75% RH, 80 °C) and anhydrous Form II THEO (blue).

At both levels of % RH and 80 °C, THEO monohydrate transitioned back to its original, anhydrous starting material within one day. Hydrate stability at 80 °C was not evident.

4.3.3.1.3. Hydrate stability at centre point conditions: 40 % RH/ 50 °C

Centre point conditions for THEO monohydrate were set up at the same time as Vial 4-A and 6-A discussed in **Section 4.3.3.1.1**, hence the weekly sampling points. **Table 4.15** shows the monohydrate lost all moisture related to its hydrated form within the first week at these conditions. TGA results for this sample (**Figure 4.46**) showed only degradation like the end point sample of ‘Medium’ crystals in Vials 4-A (**Figure 4.38**), 4-C (**Figure 4.42**) and 6-C (**Figure 4.44**). XRPD analysis of the end point sample of Vial 5-B is shown in **Figure 4.47** and clearly shows the transition back to the Form II CBZ anhydrous starting material.

Table 4.15: TGA % weight loss values for ‘Medium’ sized THEO monohydrate crystals at 40% RH and 50 °C.

Vial #	Start	Wk 1
% Weight loss (water of hydration)		
5-B (40% RH, 50 °C)	8.34	0.00

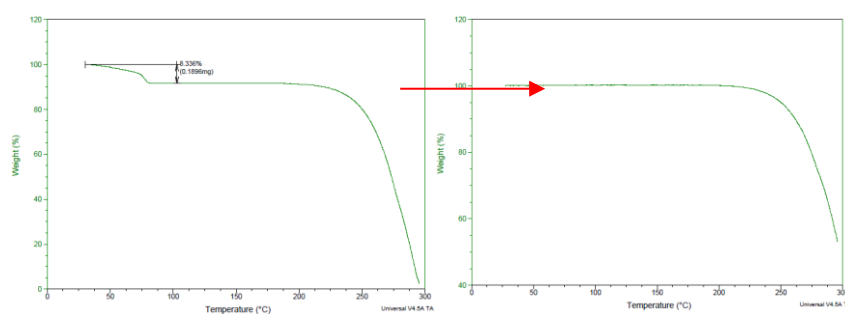


Figure 4.46: TGA analyses for ‘Medium’ THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 5-B (40% RH, 50 °C).

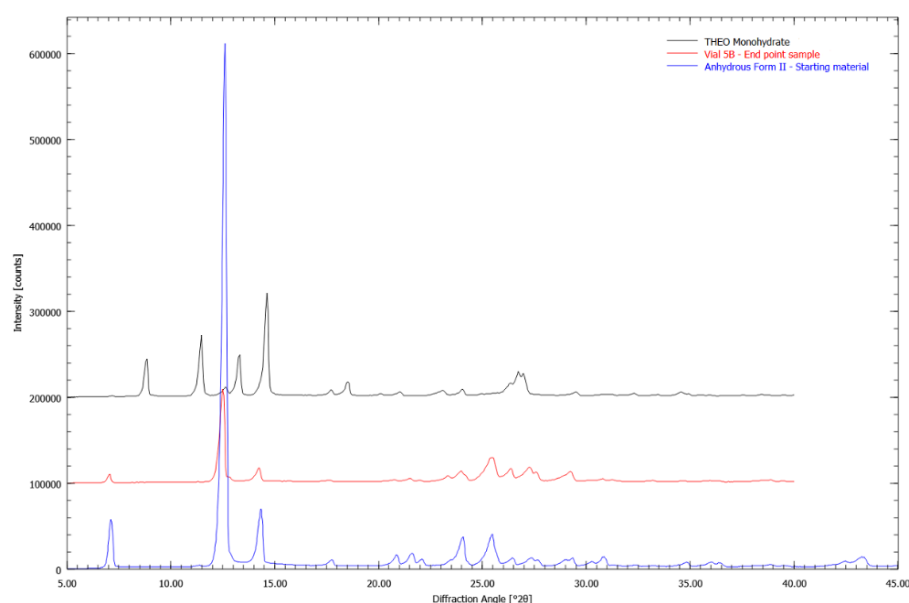


Figure 4.47: XRPD overlay for ‘Medium’ THEO monohydrate crystals showing the THEO monohydrate (black), end point sample (red) for Vial 5-B (40% RH, 50 °C) and anhydrous Form II THEO (blue).

4.3.3.2. Effects of temperature on hydrate stability of THEO monohydrate generated from the standard method (Medium sized particles)

In this section, the effects of temperature on hydrate stability 'Medium' CBZ monohydrate particles are examined, comparing:

- Vial 4-A (25 °C/2% RH) vs. Vial 4-C (80 °C/2% RH) (**Table 4.12**)
- Vial 6-A (25 °C/2% RH) vs. Vial 6-C (25 °C/2% RH) (**Table 4.12**)

Table 4.16 shows the comparison of TGA % weight loss values across the sampling periods for Vial 4-A and Vial 4-C, at 2% RH. Both samples reached 0.00% weight loss within their first respective sampling points. Due to differences in sampling points, comparing these samples is somewhat difficult. However, as Vial 4-C had a value of 0.00% after only one day, this can be assumed to also be the case after one week. TGA graphs of both showed only degradation (**Figure 4.38** and **Figure 4.42**) and, as previously indicated, XRPD analysis (**Figure 4.39** and **Figure 4.43**) showed that both end point samples had all the peaks corresponding to the anhydrous Form II THEO.

Table 4.16: TGA % weight loss values for 'Medium' sized THEO monohydrate crystals at different temperatures at 2% RH.

Vial #	Start	Wk 1
% Weight loss (water of hydration)		
4-A (2% RH, 25 °C)	8.34	0.00
	Start	Day 1
4-C (2% RH, 80 °C)	8.34	0.00

From the findings, it shows that 2% RH is too low a relative humidity to provide an environment which was conducive for hydrate stability. Under both temperatures discussed in this work, the dihydrate was lost.

Table 4.17 shows the comparison of TGA % weight loss values for Vial 6-A and Vial 6-C, showing the effects of temperature at 75% RH. Hydrate stability is only seen at the lower temperature of 25 °C, up to the 12-week period. TGA results confirmed these findings with Vial 6-A showing the step-like feature and % weight loss associated with the dihydrate (**Figure 4.40**) while the result for Vial 6-C (**Figure 4.44**) showed only degradation. Confirmation was further provided by XRPD analysis (**Figure 4.41** and **Figure 4.45**) whereby ‘Medium’ crystals of Vial 6-A overlaid well with the monohydrate form while Vial 6-C end point sample overlaid with the anhydrous Form II THEO.

Table 4.17: TGA % weight loss values for ‘Medium’ sized THEO monohydrate crystals at different temperatures at 75% RH.

Vial #	Start	Wk 12
<i>% Weight loss (water of hydration)</i>		
6-A (75% RH, 25 °C)	8.34	7.74
	Start	Day 1
6-C (75% RH, 80 °C)	8.34	0.00

At 75% RH, hydrate stability was found at 25 °C however it was lost at 80 °C in one day, suggesting the higher temperature increased the rate of dehydration. Like CBZ dihydrate, this information can be used to build a hydrate stability design space which could be used to prepare a future drying study plan.

4.3.3.3. Overall hydrate stability dependence on humidity and temperature for THEO monohydrate generated from the standard method (Medium sized particles)

Figure 4.48 shows the full overlay of all end point samples along with the initial monohydrate trace and the anhydrous Form II THEO. Vial 6-A was the only sample to overlay with the monohydrate pattern meaning the conditions of 75% RH and 25 °C allowed for hydrate stability over the 12-week period for THEO monohydrate. All other XRPD traces of end point samples (Vial 4-A, 5-B, 4-C and 6-C) contained the peaks of the anhydrous Form II THEO.

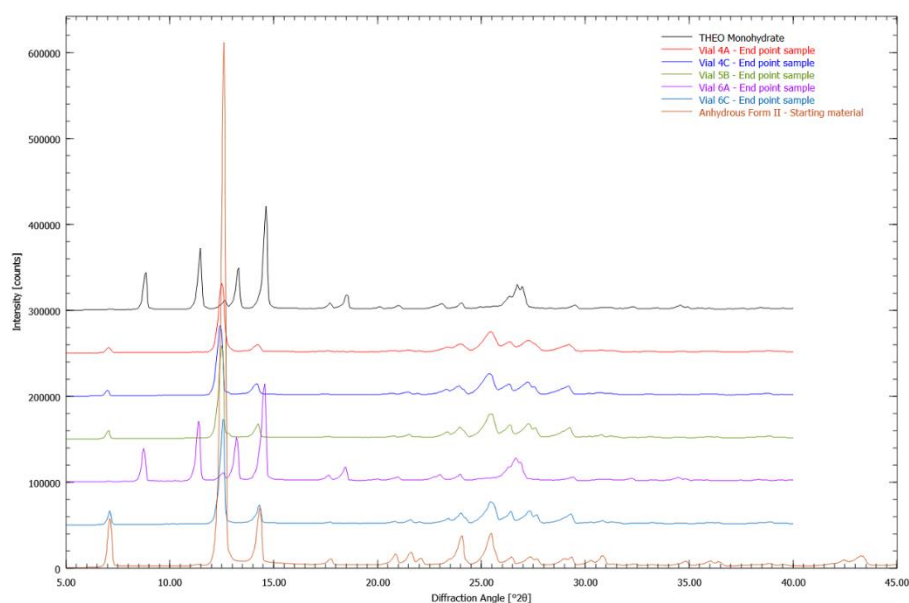


Figure 4.48: XRPD overlay for ‘Medium’ THEO monohydrate crystals showing the THEO monohydrate (black), end point samples of Vial 4-A (red), Vial 4-C (dark blue), Vial 5-B (green), Vial 6-A (purple), Vial 6-C (light blue) and anhydrous Form II THEO (dark orange).

Figure 4.49 provides a summary of the hydrate stability study results showing the form of the end point samples recovered. This will be an important tool when putting together a study plan for drying study experiments.

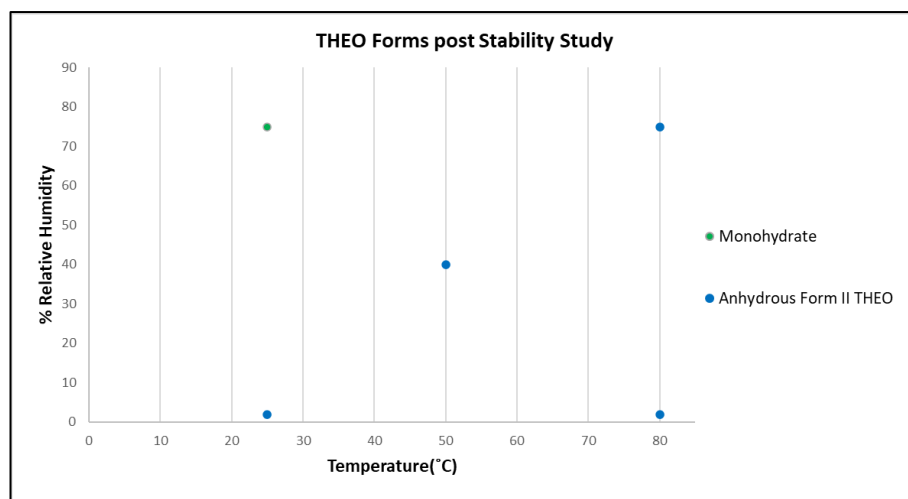


Figure 4.49: Solid-state form diagram for THEO end point samples, post Stability Study using the Cuspor Ageing System.

4.3.3.4. Effects of PSD on hydrate stability of THEO monohydrate

At the start of **Section 4.3.2**, it was discussed that each vial contained portions of crystals with differing PSD. By comparing the TGA % weight loss values for each sample within each vial, the effects of PSD at those specific conditions were also investigated.

4.3.3.4.1. Effects of PSD on hydrate stability at 2% RH, 25 °C

From **Table 4.18**, results showed that hydrate stability for each of the samples was lost when held at 2% RH and 25 °C for one week. The level of dehydration within this sampling period appeared to be unaffected by differences in particle size. TGA analyses of ‘Small’, ‘Medium’ and ‘Large’ crystals showed only degradation for each end point sample, see **Figure 4.50**, **Figure 4.38** and **Figure 4.51** respectively). From XRPD analysis, **Figure 4.52**, each sample contained all peaks relating to the anhydrous Form II THEO. There were no peaks indicative of the monohydrate present.

Table 4.18: TGA % weight loss values for THEO monohydrate crystals of increasing PSD at 2% RH and 25 °C (Vial 4-A).

Vial 4-A	Start	Wk 1
% Weight loss (water of hydration)		
Small	8.94	0.00
Medium	8.34	0.00
Large	9.54	0.00

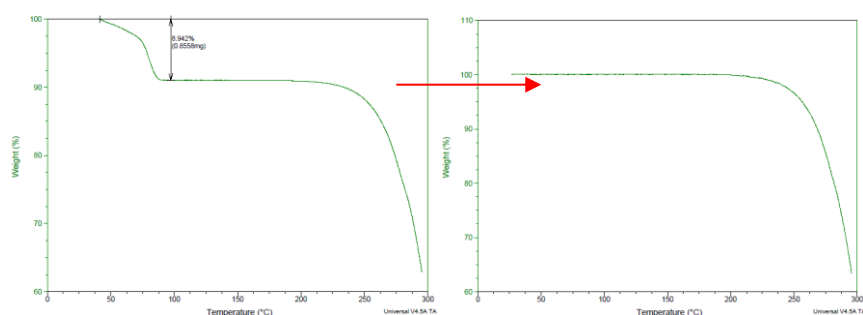


Figure 4.50: TGA analyses for 'Small' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-A (2% RH, 25 °C).

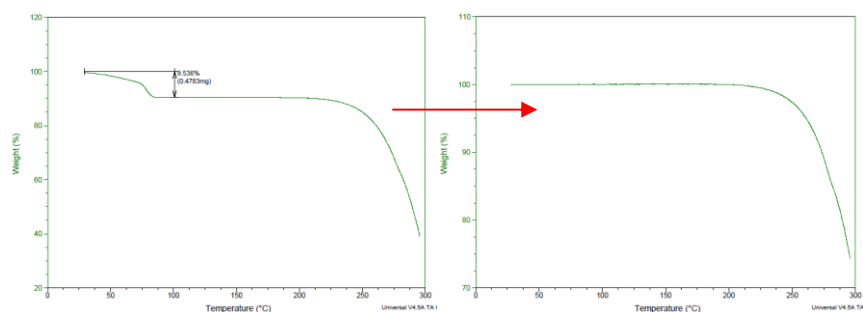


Figure 4.51: TGA analyses for 'Large' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-A (2% RH, 25 °C).

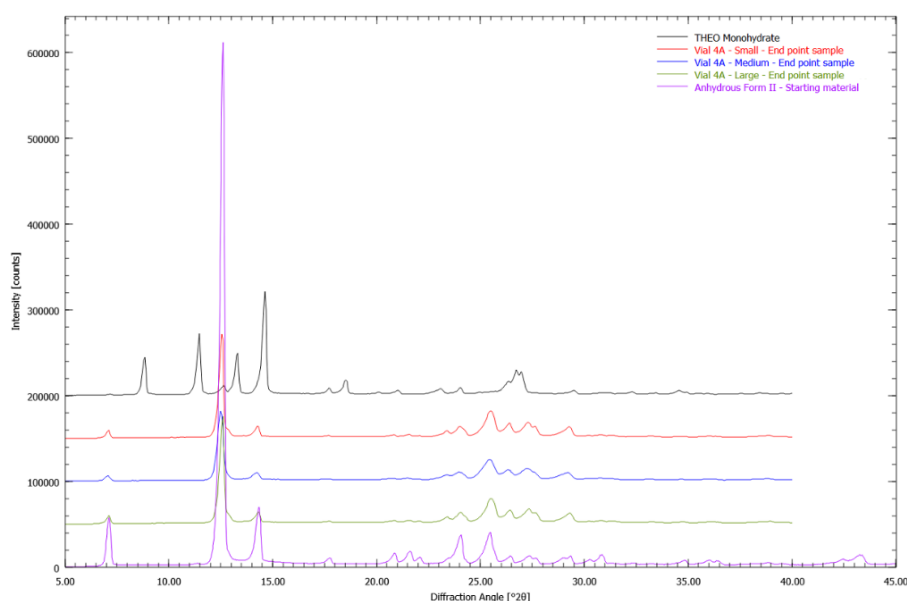


Figure 4.52: XRPD overlay for THEO monohydrate (black), end point samples of 'Small' (red), 'Medium' (dark blue), 'Large' (green) THEO crystals from Vial 4-A and the anhydrous Form II THEO (purple).

Changing PSD of THEO monohydrate crystals does not provide protection against dehydration of the hydrate form when held at 2% RH and 25 °C.

4.3.3.4.2. Effects of PSD on hydrate stability at 75% RH, 25 °C

Table 4.19 showed that hydrate stability across each of the samples was maintained when held at 75% RH and 25 °C for up to a 12-week period. Differences in TGA % weight loss values from the theoretical value of ~9.08% was likely due to the instrument equilibrating prior to the analysis beginning. TGA analyses of 'Small', 'Medium' and 'Large' crystals showed the step-like feature with a % weight loss close to the expected value for each end point sample, (see **Figure 4.53**, **Figure 4.40** and **Figure 4.54** respectively). From XRPD analysis, **Figure 4.55**, each sample trace resembled that of the monohydrate form after the 12 weeks.

Table 4.19: TGA % weight loss values for THEO monohydrate crystals of increasing PSD at 75% RH and 25 °C (Vial 6-A).

Vial 6-A	Start	Wk 1	Wk 2	Wk 4	Wk 8	Wk 10	Wk 12
<i>% Weight loss (water of hydration)</i>							
Small	8.94	9.00	9.12	9.02	8.99		9.06
Medium	8.34	8.83	8.82	8.88	8.45		7.74
Large	9.54	9.09	8.99	9.08	8.99		9.20

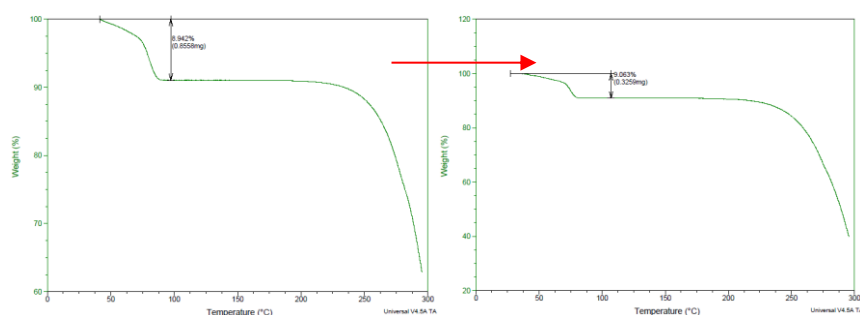


Figure 4.53: TGA analyses for 'Small' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-A (75% RH, 25 °C).

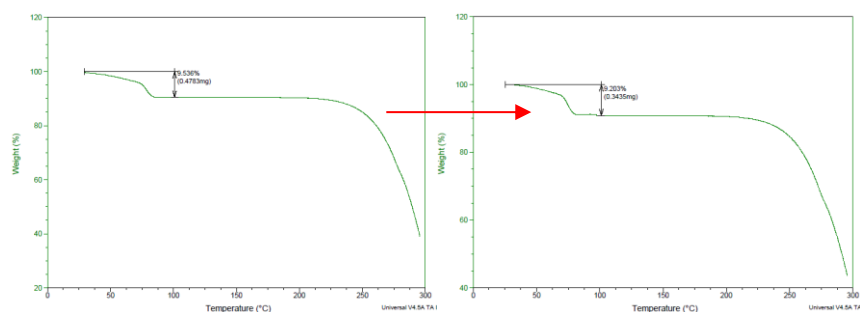


Figure 4.54: TGA analyses for 'Large' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-A (75% RH, 25 °C).

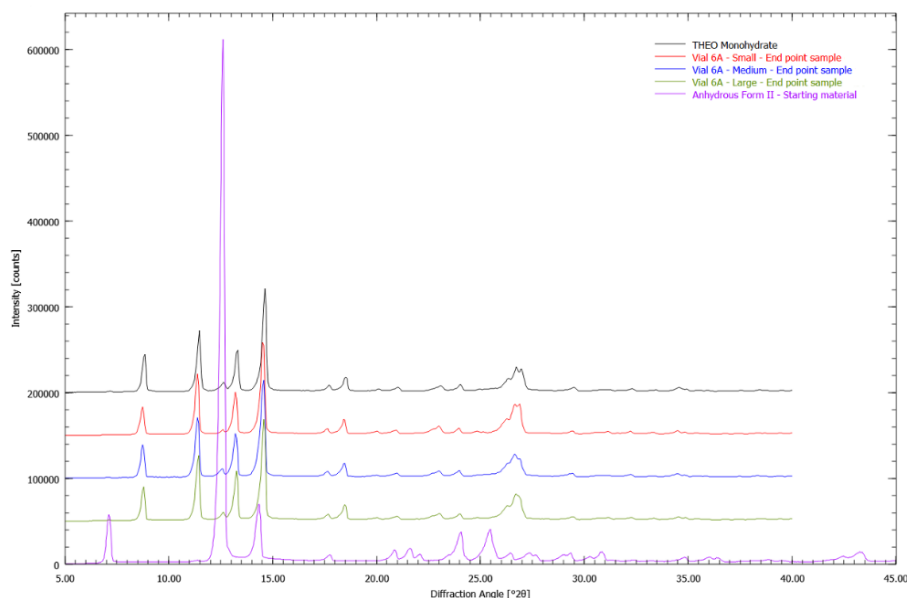


Figure 4.55: XRPD overlay for THEO monohydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) THEO crystals from Vial 6-A and the anhydrous Form II THEO (purple).

While this set of conditions showed significant hydrate stability, differences due to PSD were not found. THEO monohydrate has great stability at these conditions regardless of particle size.

4.3.3.4.3. Effects of PSD on hydrate stability at 40% RH, 50 °C

From **Table 4.20**, results showed that hydrate stability at 40% RH and 50 °C, for all crystals was lost within one week. Once again, differences in dehydration and dehydration levels due to PSD was not seen. TGA analyses showed only degradation after one week at these conditions (see **Figure 4.56**, **Figure 4.46** and **Figure 4.57** respectively). From XRPD analysis, (**Figure 4.58**) end points of each sample contained all peaks relating to the Form II THEO anhydrous starting material.

Table 4.20: TGA % weight loss values for THEO monohydrate crystals of increasing PSD at 40% RH and 50 °C (Vial 5-B).

Vial 5-B	Start	Wk 1
% Weight loss (water of hydration)		
Small	8.94	0.00
Medium	8.34	0.00
Large	9.54	0.00

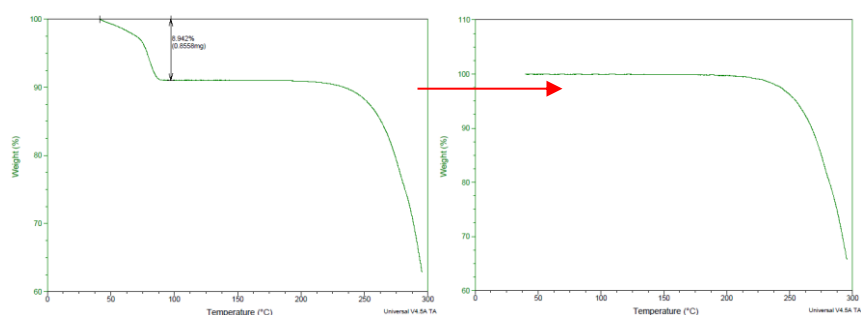


Figure 4.56: TGA analyses for 'Small' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 5-B (40% RH, 50 °C).

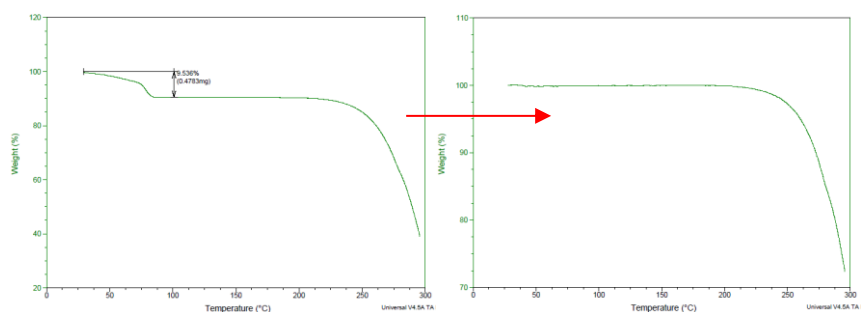


Figure 4.57: TGA analyses for 'Large' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 5-B (40% RH, 50 °C).

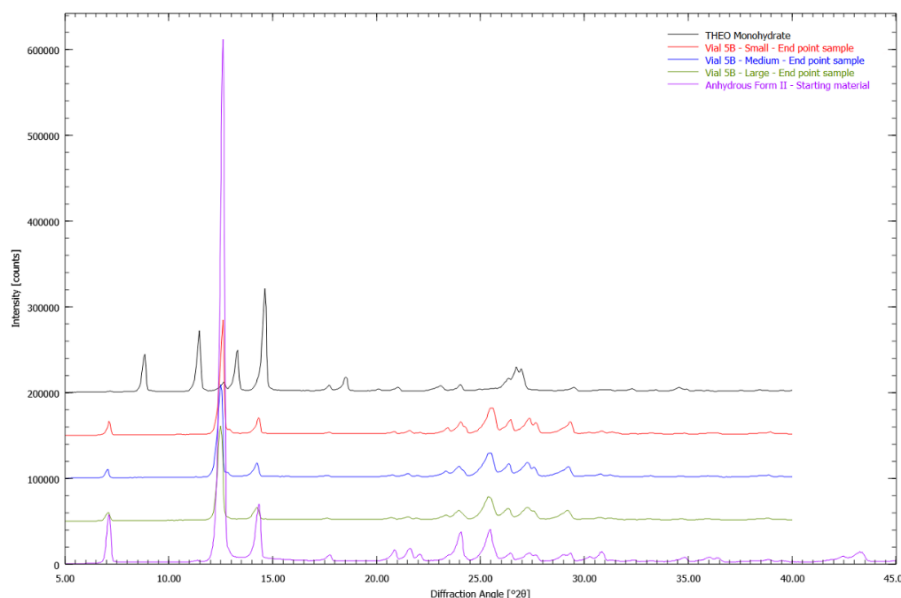


Figure 4.58: XRPD overlay for THEO monohydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) THEO crystals from Vial 5-B and the anhydrous Form II THEO (purple).

In keeping with the previous two sections, differences in dehydration due to the PSD of THEO monohydrate crystals were not found.

4.3.3.4.4. Effects of PSD on hydrate stability at 2% RH, 80 °C

From **Table 4.21**, results showed that hydrate stability across each of the samples was lost when held at 2% RH and 80 °C for one week. Differences in PSD did not impact the level of dehydration within the sampling period. TGA analyses of ‘Small’, ‘Medium’ and ‘Large’ crystals showed only degradation for each end point sample (see **Figure 4.59**, **Figure 4.42** and **Figure 4.60** respectively). XRPD analysis (**Figure 4.61**) showed end point samples of each contained the peaks relating to the Form II THEO anhydrous starting material. Peaks of the monohydrate form were not present.

Table 4.21: TGA % weight loss values for THEO monohydrate crystals of increasing PSD at 2% RH and 80 °C (Vial 4-C).

Vial 4-C	Start	Day 1
% Weight loss (water of hydration)		
Small	8.94	0.00
Medium	8.34	0.00
Large	9.54	0.00

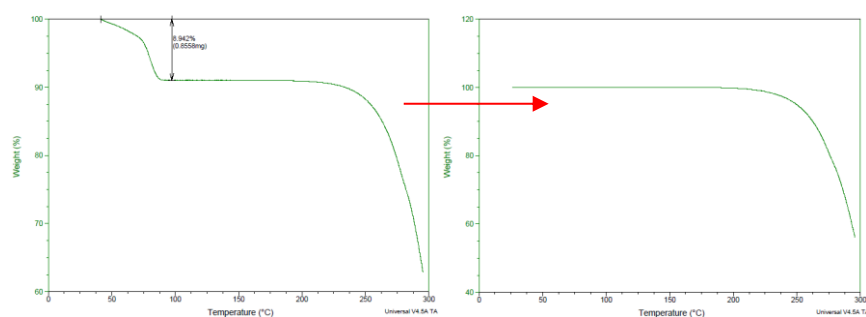


Figure 4.59: TGA analyses for 'Small' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-C (2% RH, 80 °C).

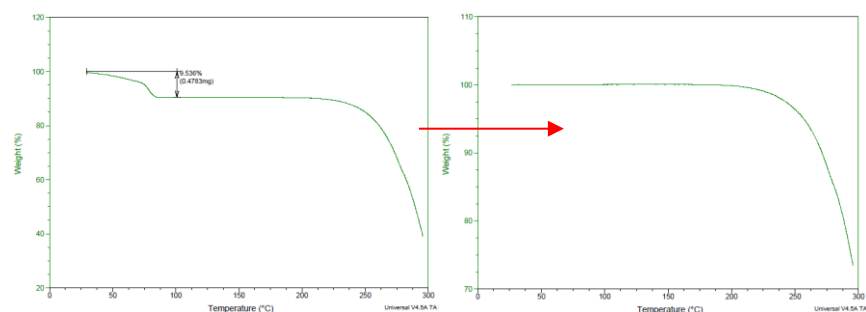


Figure 4.60: TGA analyses for 'Large' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 4-C (2% RH, 80 °C).

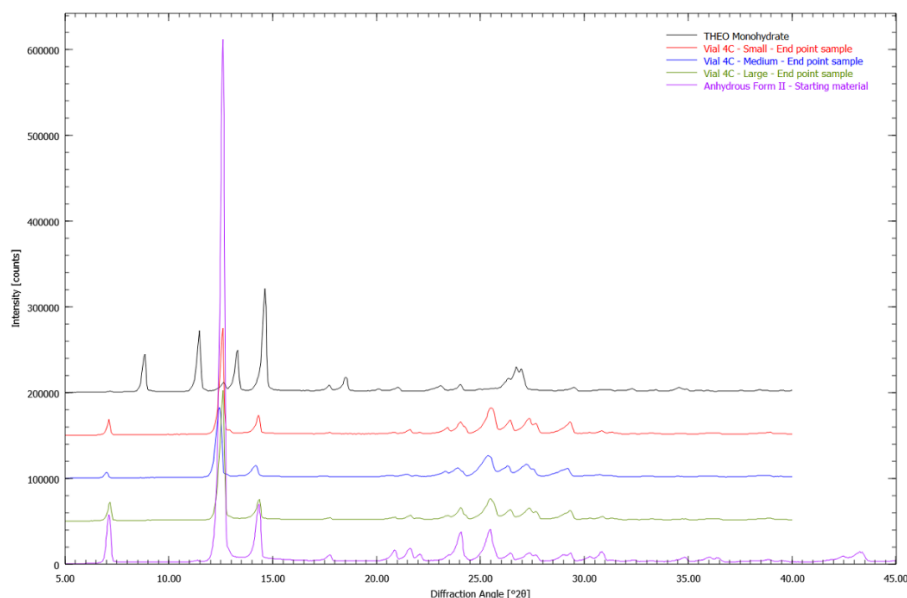


Figure 4.61: XRPD overlay for THEO monohydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) THEO crystals from Vial 4-C and the anhydrous Form II THEO (purple).

At 2% RH and 80 °C, hydrate stability was not seen after one day for each of the THEO samples, regardless of increasing PSD.

4.3.3.4.5. Effects of PSD on hydrate stability at 75% RH, 80 °C

Table 4.22 summarises the results for each sample when held at 75% RH and 80 °C which showed that hydrate stability was lost after one week. Differences in PSD did not impact the dehydration within the sampling period. TGA analyses of ‘Small’, ‘Medium’ and ‘Large’ crystals showed only degradation for each end point sample (see **Figure 4.62**, **Figure 4.44** and **Figure 4.63** respectively). XRPD analysis (**Figure 4.64**) showed each diffractogram overlaid well with the anhydrous Form II THEO, with no common peaks of the monohydrate form.

Table 4.22: TGA % weight loss values for THEO monohydrate crystals of increasing PSD at 75% RH and 80 °C (Vial 6-C).

Vial 6-C	Start	Wk 1
% Weight loss (water of hydration)		
Small	8.94	0.00
Medium	8.34	0.00
Large	9.54	0.00

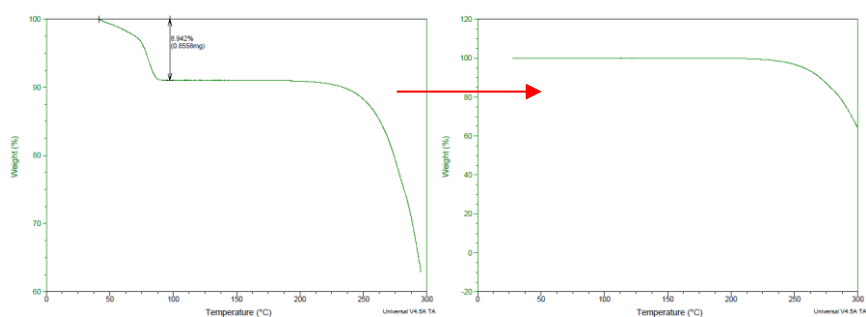


Figure 4.62: TGA analyses for 'Small' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-C (75% RH, 80 °C).

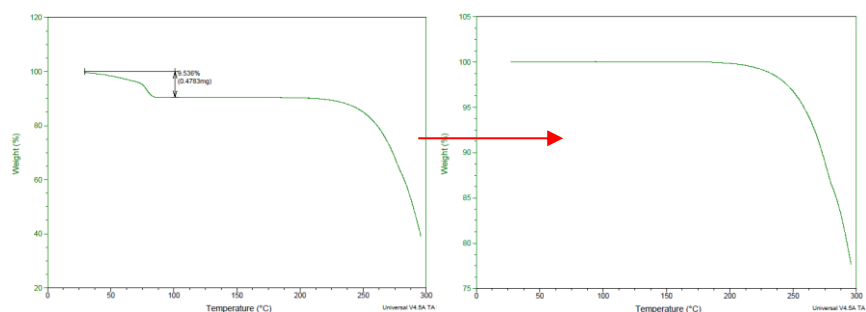


Figure 4.63: TGA analyses for 'Large' THEO monohydrate crystals showing the start (left) and end (right) point samples for Vial 6-C (75% RH, 80 °C).

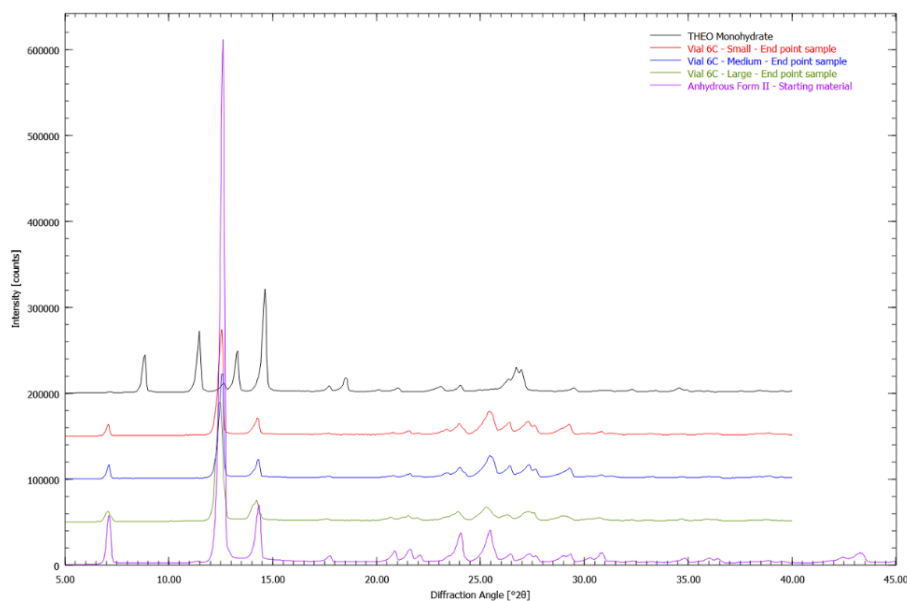


Figure 4.64: XRPD overlay for THEO monohydrate (black), end point samples of ‘Small’ (red), ‘Medium’ (dark blue), ‘Large’ (green) THEO crystals from Vial 6-C and the anhydrous Form II THEO (purple).

Once again, differences in PSD did not result in determinable differences in dehydration at these conditions.

4.4. Chapter Conclusions

For ‘Medium’ CBZ dihydrate samples, Vial 3-A, at 75% RH and 25 °C, was the only sample to retain its hydrate form and have TGA results and XRPD traces which matched the dihydrate form. Not only was this the only sample to have the dihydrate present after the start of the experiment but the stability of the dihydrate form was consistently seen across a 12-week period. All other XRPD traces of end point samples (Vial 1-A, 2-B, 1-C and 3-C) contained the peaks for the Form III CBZ anhydrous starting material. The only other difference was the peak at 9.40° 2θ and 12° 2θ in the trace for Vial 1-A (2% RH and 25 °C). The peak at 9.40° 2θ suggests the presence of the anhydrous Form I CBZ, while the peak at 12° 2θ is a peak characteristic of the dihydrate. Regardless of temperature, 2% RH

was too low for hydrate stability and at the higher temperatures of 50 °C and 80 °C, water of hydration was lost.

Effects of PSD on CBZ dihydrate dehydration were noted in Vial 3-A (75% RH and 25 °C) and Vial 2-B (40% RH and 50 °C). At 75% RH and 25 °C, while TGA results indicate that the hydrate was retained, XRPD results showed the start of 'Small' and 'Large' crystals transitioning to the Form III CBZ anhydrous material, while for 'Medium' crystals, the dihydrate form was retained throughout the 12-week period. For the centre point conditions of 40% RH and 50 °C, TGA scans for 'Large' crystals differed to those of the 'Small' and 'Medium' crystals. Water of hydration in the 'Large' crystals took longer to be fully removed, suggesting more time was required for the moisture to travel through the longer channels to exit the crystal lattice. While PSD did affect the dehydration in two of the vials, neither effect was enough to protect the hydrate form from dehydration.

Examining 'Medium' THEO monohydrate crystals, hydrate stability was only found within one vial and that was Vial 6-A (75% RH, 25 °C). These were the same conditions for which stability was found for CBZ dihydrate. Under these conditions, XRPD results showed the monohydrate pattern being present for the entire 12-week period. All other conditions resulted in the loss of the hydrate form within the first sampling period, after one day for 80 °C sample and one week for 25 °C and 50 °C samples. The loss of the hydrate form resulted in all samples transitioning back to the anhydrous Form II THEO. From the results, it showed that 2% RH is too low of a relative humidity for hydrate stability and the higher temperature of 50 °C and 80 °C also negatively impacted the hydrate form. In contrast to the CBZ dihydrate samples, there was no set of conditions where differences in the PSD of the crystals resulted in differences in the dehydration of the samples. Where the hydrate form was lost, it

was lost across each of the 'Small', 'Medium' and 'Large' portions of the monohydrate and the same can be said for the vial where the monohydrate remained stable; each sample behaved the same.

While similarities between both compounds were found through this work, form boundaries are unique to a compound and understanding the stability relationship between the anhydrous and hydrate form is critical for developing a drying protocol. Ultimately from this work, it was found that the stability of the hydrate was affected by both temperature and the % RH levels. It is hoped that this work will be used to help inform drying studies inside a lab oven. For this, targeting vapour pressure levels might be more beneficial than % RH. The oven setup involves targeting a % RH level on the outside which is then affected by the temperature, oven pressure and dimensions inside the oven which can alter the % RH being experienced by the sample inside. Increasing temperatures causes an increase in the kinetic energy of the molecules moving into the vapour phase which in turn, increases the vapour pressure. This ability for more water molecules to transition into the vapour phase is the reason % RH decreases with higher temperature.

A downside to this work was the invasive sampling approach, so having access to real time information during the dehydration would build a better picture. From this work, it was decided to use the DVS to collect both dehydration and drying data for both compounds. This instrument allows for changes to the sample weight to be tracked, in real time and produces a small amount of sample at the end for off-line solid-state analysis. It was hoped this would provide further insights into the drying and dehydration kinetics of both CBZ dihydrate and THEO monohydrate.

CHAPTER 5. Drying Studies using the DVS Intrinsic Apparatus

5.1. Abstract

The DVS Intrinsic was successfully used for small scale drying studies on two model hydrate compounds. It provided a highly controlled environment and recorded data every minute, allowing for repeatable drying curves, with numerous data points, to be plotted along with the collection of drying and dehydration kinetics data.

An initial experiment, involving CBZ dihydrate being held at 40 °C and 0% RH produced a graph of moisture content versus time with an inflection point which separated sections of the curve with differing slopes. M.C. calculations, based on the dry weights of the hydrated form showed the point of inflection to fall at 0% M.C. The hypothesis was that % M.C. greater than 0% related to excess loosely bound moisture, while at 0% M.C., the dry hydrated form was present, and where values of % M.C. were less than 0%, this was indicative of the loss of water of hydration.

DVS experiments where the end point sample was suspected to be the dry hydrate form (0% M.C.) coupled with off-line analytical techniques, confirmed that the point of inflection in the moisture content versus time profile did denote changes in the type of moisture being removed, and that the hypothesis was correct. Different regions of the drying profiles obtained were identified and attributed to excess, loosely bound moisture being removed first, followed by a period of (dry) hydrate stability and then the water of hydration being removed. Using the calculations for % M.C. and sample weights from the start and end of each experiment, drying rates were calculated and plotted. This gave

information on constant drying rate periods and falling rate periods for drying of both hydrate compounds (CBZ dihydrate and THEO monohydrate).

For CBZ dihydrate, under conditions of increasing % RH, the time taken for the removal of loosely bound moisture increased, ranging from ~6 min at low RH levels to ~52 min at 80% RH. Increasing the temperature reduced drying times. Stability of the dry CBZ dihydrate was found in environments $\geq 40\%$ RH, with the loss of the water of hydration being induced only by using a 0% RH step immediately after the set desired RH conditions. Below 40% RH, hydrate stability was minimal, lasting <10 min at 10% RH and 20% RH, after which, dehydration began. Dehydration was found to occur gradually at these lower % RH environments, often with all remaining moisture (water of hydration) being fully removed prior to the 0% RH step starting. For the higher RH values, once the 0% RH period began, dehydration took approximately 30 minutes. The original anhydrous Form III starting material remained after drying under these conditions. At 40% RH and above, an XRPD pattern which differed from that of the anhydrous Form III was observed, indicating differences in crystal structure between the dehydrated CBZ, the dihydrate form and the anhydrous starting material from which the dihydrate was prepared.

THEO monohydrate was investigated in a similar study, with a point of inflection being observed, similarly to CBZ dihydrate, for samples held at 40 °C and 0% RH. The drying study showed that an increase in % RH resulted in longer drying times for removal of loosely bound moisture (to get to the “dry” hydrate), from 5 min at 0% RH to 19 min at 80% RH. Results also showed that increased temperatures gave shorter drying times. Consistent with this observation was the fact that calculated drying rates decreased with increasing % RH environments. At the highest levels of % RH, drying times (for

removal of loosely bound moisture) were shorter than for CBZ dihydrate. This is likely due to the higher initial % moisture content values of the CBZ dihydrate samples (average value being 47.5%) over THEO monohydrate samples (average values being 30.0%). All other conditions can be directly compared and as this relates to unbound moisture, the structure and intermolecular forces between the compounds are not at play. The other difference between the two compounds relates to the amount of moisture being removed during drying. Significant periods of dry hydrate stability were seen for THEO monohydrate samples at 40% RH and above. Stability was only disrupted after a 0% RH step began, which induced dehydration. At 0 and 10 % RH levels, only a point of inflection detailed the start of dehydration while at 20% RH, a short 10 min period of hydrate stability preceded dehydration.

Both CBZ dihydrate and THEO monohydrate are stoichiometric channel hydrates, with their stoichiometric amounts of water being confirmed during this period of stability. Both hydrates show similar stability in environments $\geq 20\%$ RH. Below this, THEO monohydrate showed more sensitivity to the low % RH levels. This could be due to sample size and the level of exposure to the conditions, as CBZ dihydrate is known to agglomerate upon drying which could have provided a small amount of protection, allowing ~ 10 min of hydrate stability at 10% RH. Similar to CBZ, the water of hydration of THEO monohydrate was completely removed prior to the 0% step for samples analysed at $<40\%$ RH. Dehydration of the monohydrate, after drying at higher % RH levels took approximately 100 minutes, which is significantly longer than the CBZ samples.

Drying, dehydration and transitions were also examined using a non-ambient chamber attachment to an XRPD instrument. This allowed for more information to be gathered of form transitions

while water was leaving the surface and from within the crystal lattice. It correctly saw the transition from CBZ dihydrate to an amorphous form due to the breaking of the amide C=O bond ^[118], followed by the rearrangement of the lattice to give the Form III starting material. For THEO monohydrate, non-ambient XRPD analysis showed that dehydration began with the change from monohydrate to metastable anhydrate once loosely bound moisture was removed. It is reported in the literature that keeping the metastable anhydrate at these conditions would result in a transition to the Form II starting material, but this was not seen during the current work. ^[119] For both hydrate samples, increasing % RH environments gave longer drying times, while at the same % RH, an increase in temperature resulted in shorter drying times. Periods of hydrate stability were found for both compounds, meaning a time point where the dry hydrated forms are present can be determined and obtained.

5.2. Introduction

The main aim of the studies presented in this chapter, was to develop a highly controlled drying environment to allow drying data to be gathered in order to generate drying curves and enable understanding of drying and dehydration kinetics for API hydrates. It was hoped that the initial prototype experimental setup would include the following:

- Temperature control
- Pressure control
- % RH tracking/control
- Sample weight measurement

Results from DVS analysis have previously been discussed in **Sections 3.3.1.1** and **3.3.1.2**, with regards to sorption and

desorption behaviour of both API hydrate forms and starting materials. Further investigation into the raw data generated by the DVS showed that several parameters were successfully being monitored/tracked and to a high degree of control. The DVS Intrinsic offered the following parameters for control:

- Temperature control (20-40 °C)
- Gas inlet flow rate (set at 2 bar)
- % RH tracking/control (0-98% RH)
- Sample weight tracking via the UltraBalance™

A quick comparison of the two lists above raised the following question: could the DVS be used as a small-scale dryer, within the operational limits of the instrument?

Commonly, the DVS is used for the measurement of vapour sorption/desorption phenomena. Like the TGA, small amounts of sample are loaded into a pan which is suspended onto a hangdown wire. Using a mass flow controller, air with accurately known % relative humidity is passed over the sample at a set temperature and fixed gas inlet flow rate. The hangdown wire is attached to a microbalance. Central to each Surface Measurement Systems DVS instrument, is their trademarked Ultrabalance™ technology. This provides ultra-sensitive recording of sample masses. Traditional DVS experiments can incorporate significant changes to conditions and reaching equilibrium at each step can take minutes, hours, or days.^[120] The microbalance shows very good long-term stability for such experiments.

Initial brainstorming and considerations around developing a new drying setup/configuration revolved around a weight tracking capability. Tracking sample weight throughout the drying process was deemed essential to develop a drying platform which is data

driven and comes from a place of knowledge. The hangdown wire approach has been successfully documented previously ^[51] however, it proved difficult to replicate in the lab in a standard closed oven environment.

The DVS offered a suitable alternative for weight tracking, albeit on a smaller scale. Couple this with the schematic of the DVS Intrinsic, shown below **Figure 1.5**, and the makings of a highly controlled, small-scale drying environment were evident. Several operation limits that had to be worked within were identified, e.g. the 20-40 °C temperature range, small sample sizes, set gas inlet flow rate and gas inlet pressure.

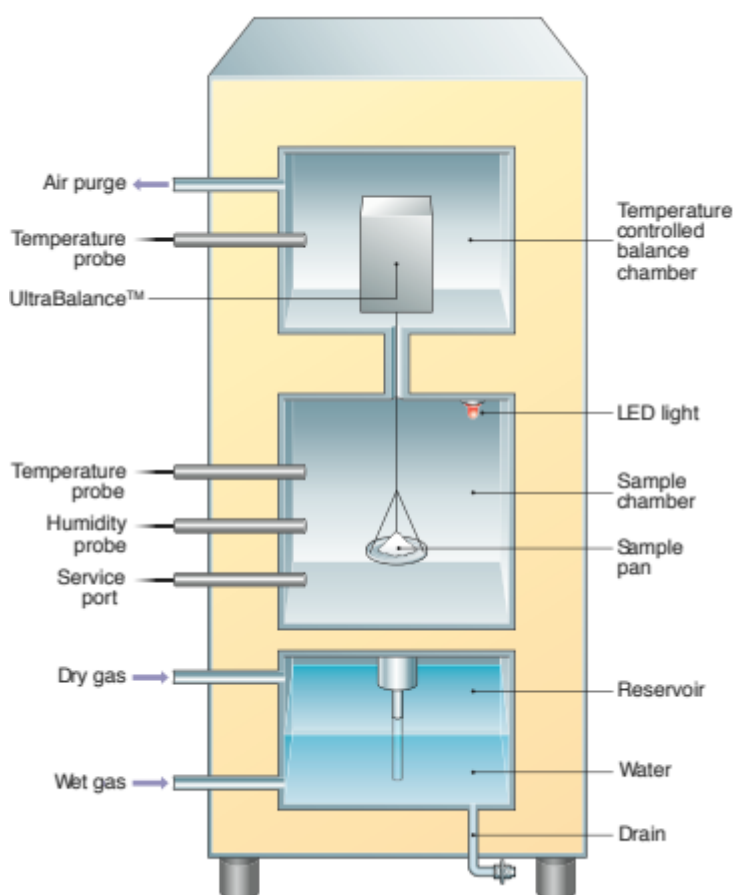


Figure 5.1: Schematic of the DVS Intrinsic.

The main aim in developing most drying processes is to recover the desired sample form in the shortest drying time, therefore, using less energy and saving time and money. In some cases, it may be necessary to remove excessive levels of moisture to guarantee a certain level of purity or morphology or, in this case of this work, provide a level of control on parameters which can protect the desired sample from over-drying and dehydration of a hydrate sample. Parameter control is critical. A parameter of particular interest within a drying platform is humidity, whether the API has hygroscopic properties, or the hydrated form is the desired product. At manufacturing scale, significant variations in humidity could affect the extent of drying of the material and consequently the purity and form, which is a huge concern from a quality and safety perspective. Through the work outlined in this chapter, the use of a DVS as a valuable drying screening tool for gathering comprehensive drying data with minimal sample and minimal time is demonstrated.

5.3. Results and Discussion

For the materials and methods used for this chapter, please see **Section 2.8**.

5.3.1. CBZ Dihydrate

After successful crystallisation using the standard method (**Section 2.3.1.2**), a sample with a total moisture content of ~45% was decided on as an appropriately wet starting sample. The % total Moisture Content (% M.C.) was quickly determined via the Mettler Toledo Moisture Analyzer (**Section 2.5.2**). Analysis was performed in duplicate, and results are shown in **Table 5.1** below:

Table 5.1: % Moisture Content values of wet CBZ dihydrate.

PR063- Wet CBZ Dihydrate	
S01_1 - %	48.03
S01_2 - %	46.67
<i>Average</i>	<i>47.350</i>
STDEV	0.680
% RSD	1.436

A small amount of this sample was left to dry overnight under ambient conditions of 22 °C and approximately 55% RH, to confirm the presence of the dihydrate. **Figure 5.2** below provides a summary of the solid-state analysis completed to confirm this. The DSC thermogram of the dried sample, **(A)** shows the peak of hydration at ~85 °C and the characteristic Form I melt at ~190 °C. This trace compares well with previously generated batches of CBZ dihydrate, **Section 3.3.1.1**. Similarly, TGA results, **(B)** show a weight loss of $12.93 \pm 0.07\%$ at temperatures below 100 °C which correlates well to the theoretical loss of the dihydrate being 13.22%. Actual values being lower than theoretical could be due to small water losses within the instrument under the initial conditions. Finally, XRPD analysis, **(C)** shows an overlay of the wet CBZ dihydrate and a sample of the ambiently dried CBZ dihydrate. For this work, a different XRPD instrument was used, **(Section 2.4.5)**. The instrument used in this chapter is a PANalytical Empyrean Diffractometer which allows analysis with a reduced sample size. This was advantageous for the analysis of post DVS samples which ranged from 10-20 mg. Results showed, that both patterns overlay with no additional or lost peaks for either sample. The lower intensity seen across all peaks of the ambiently dried CBZ dihydrate sample is likely to be due to differences in sample size.

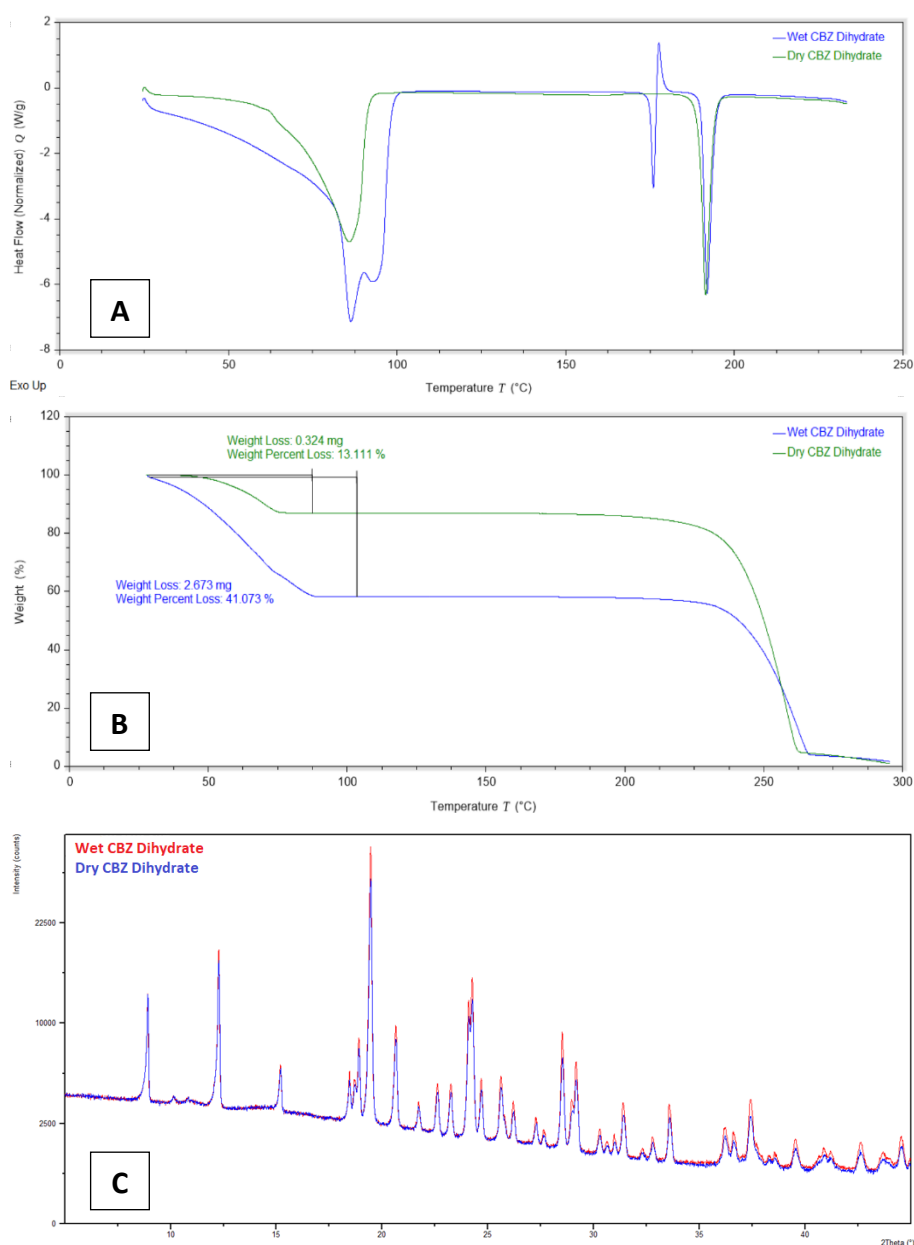


Figure 5.2: A) DSC thermogram, B) TGA, C) XRPD of the wet CBZ dihydrate (post crystallisation and filtration) and dried CBZ dihydrate (dried under ambient conditions overnight).

5.3.1.1. Effects of relative humidity on wet CBZ Dihydrate moisture content over time at 40 °C

The change in moisture content experienced by 'wet' CBZ dihydrate samples (containing excess loosely bound moisture, detailed in

Section 2.3.1.4), subjected to DVS analysis at different RH values is captured in **Figure 5.3**.

The initial phase of the profile (1) has a distinct slope and is hypothesised as being associated with the removal of excess moisture. This is followed by a region (2) of hydrate stability, where conditions allow. Here, it is suspected that the dry dihydrate form is present and under favourable conditions, remains stable until a 0% RH step is initiated. The final phase of the profile (3), with a second distinct slope, is associated with dehydration (removal of water of hydration) due to low % RH or the introduction of a 0% RH step. By starting with a sample containing excess loosely bound moisture, the DVS can give insights into the drying and dehydration kinetics, along with stability of the hydrate after drying.

To better compare results, data is shown as % change from the initial %M.C. of each sample. By adjusting data to a common starting point, changes over time in relation to different conditions are clearer to see and can be compared. The raw data showed differences in initial % M.C., with a range of 39-55% and this is likely due to differences in the starting point (sample wetness) of each experiment. Efforts to minimise these differences involved using a standardised approach for sample preparation prior to saving data. The standardised approach involved allowing enough time for the mass of each sample to be acknowledged by the instrument and once the highest mass was reached (under each set of experimental conditions) and then began to decrease, the chosen method was started, and data saved. Sample weight decreasing shows it was being affected by the conditions. This was the start of the drying process and important information to capture. The approach was dictated by the sample and the conditions, and not a timed process. It was decided as the best option to obtain the most drying data for all samples across the wide range of experimental conditions being

investigated. For example, in low % RH experiments, the actual % RH measured increased upon sample addition. The mass flow controller in the DVS, which controls and adjusts the % RH of the system, must then adjust the system to reduce the % RH to the desired levels. If a timed approach was used, it could start the experiment too early where the sample weight is being tracked based on different conditions than those set, or if started too late, the beginning of the drying data could be lost.

Values for the calculated % M.C. was determined from DVS raw is based on the dry dihydrate and was calculated using the molecular weights of both the dehydrated and hydrated forms of CBZ along with each sample's starting weight reading and the end point sample weight. Ensuring a fully dehydrated/anhydrous form at the end of each experiment was important for the correct calculations to be made. To ensure this, a 0% RH step was introduced at the end of each experiment. For CBZ dihydrate, this step was set for 120 min, as initial DVS experiments using dry CBZ dihydrate found that the water of hydration was consistently fully removed after this amount of time.

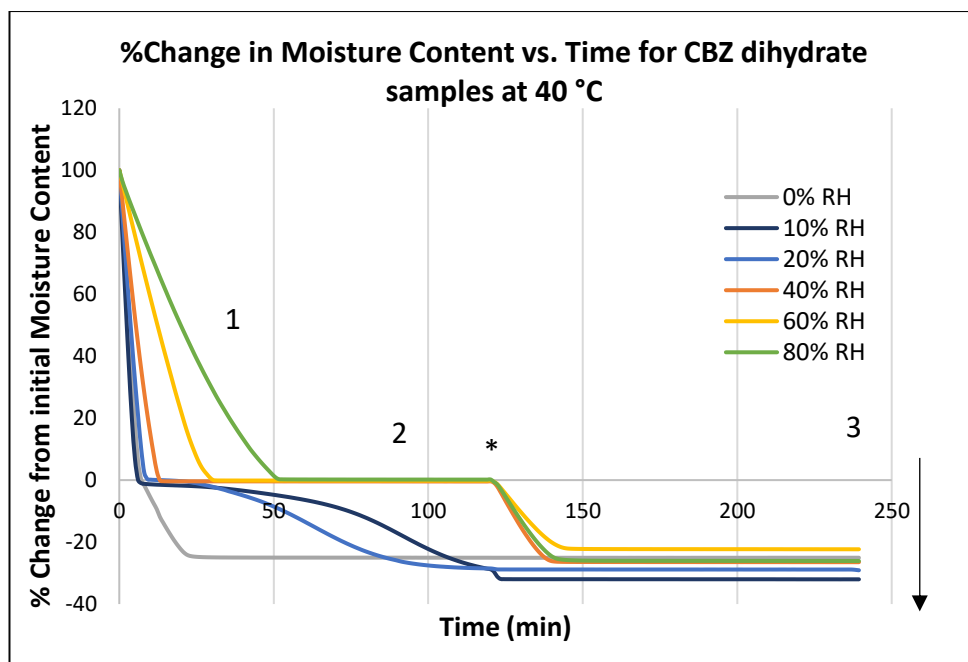


Figure 5.3: % Change in Moisture Content vs. time for CBZ dihydrate at 40 °C and different relative humidities. * denotes the start of the 0% RH step.

5.3.1.1.1. Effects of relative humidity on the drying profile of CBZ Dihydrate at 40 °C

Moisture content values >0% are believed to be the excess, loosely bound moisture. This section of each graph (shown in **Figure 5.3**) provides the drying profile of each hydrate sample. To examine more closely, **Figure 5.4** shows a zoomed in section from the original graphs' profiles shown in **Figure 5.3**.

In **Figure 5.4**, drying times are shown to increase with increasing % RH environment, except for the 0% RH sample for which the drying time is longer than at 10% RH. With air of a specific temperature, any increase in humidity levels would reduce its capacity to remove and hold additional water vapour from the sample, in turn resulting in longer drying times.^[121]

The sample which dried fastest was that held at 10% RH, with a drying time of 6 min. At 0% RH, drying was only marginally longer

at 7 min. Triplicate analysis of samples at both 0 and 10% RH, indicated that this trend in drying times was consistent. From 20% RH onwards, drying times increased as expected. At the highest level of 80% RH, drying took ~51.5 min and the protection of a wet environment is evident as the drying time is significantly longer than all other samples.

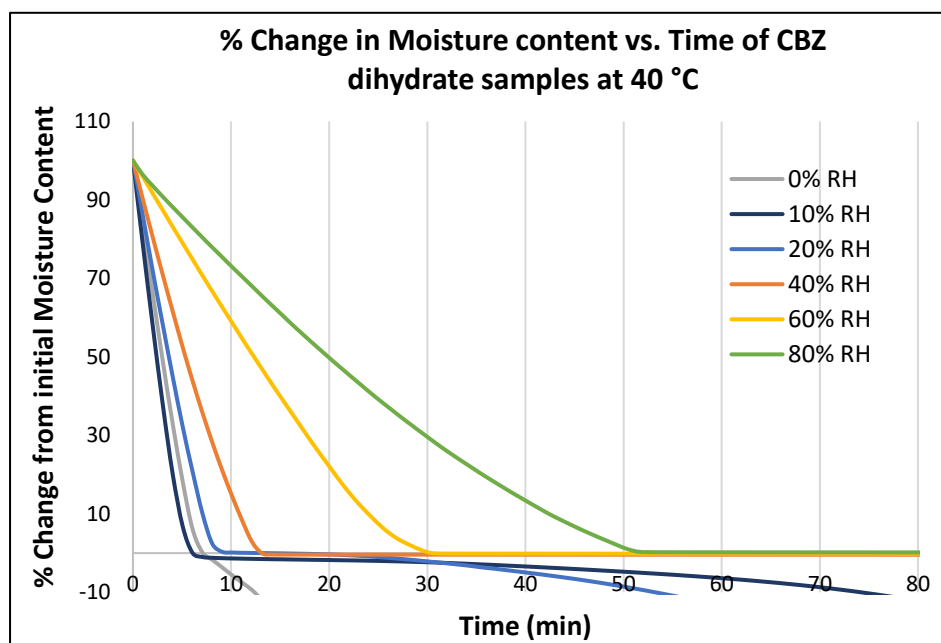


Figure 5.4: % Change in Moisture Content vs. time for CBZ dihydrate at 40 °C, showing the drying profiles of each sample at different humidities.

To examine this data in another way, **Figure 5.5** shows the traditional drying rate curves of the same samples. Reading from the right-hand side, an approximate constant rate period can be seen, where the drying rate remains constant as moisture is being lost. The point at which these changes, known as the Critical Moisture Content (CMC), is the beginning of the falling rate period. The point of inflection is more difficult to determine and shows variability across the samples. However, once the % moisture content reaches approximately 10%, a change in drying rate is evident. From this graph, it also shows that the drying rate decreases as the level of % RH increases, with the 0% RH sample

being the only one not to follow this trend, as previously discussed. This graph also shows that for samples under conditions of 10% RH and higher, each curve reaches 0% M.C., showing the periods of hydrate stability before the graphs crosses the y-axis which corresponds to dehydration taking place (<0% M.C.). The drying rate curves after this point correspond to the removal of tightly bound moisture. The smaller, gradual increase in drying rate, as seen for 10 and 20%, reflects the gradual loss of moisture seen in the previous graph, **Figure 5.3**. The sudden increase in the drying rates of the remaining samples, correspond to the start of the 0% RH step introduced, inducing dehydration.

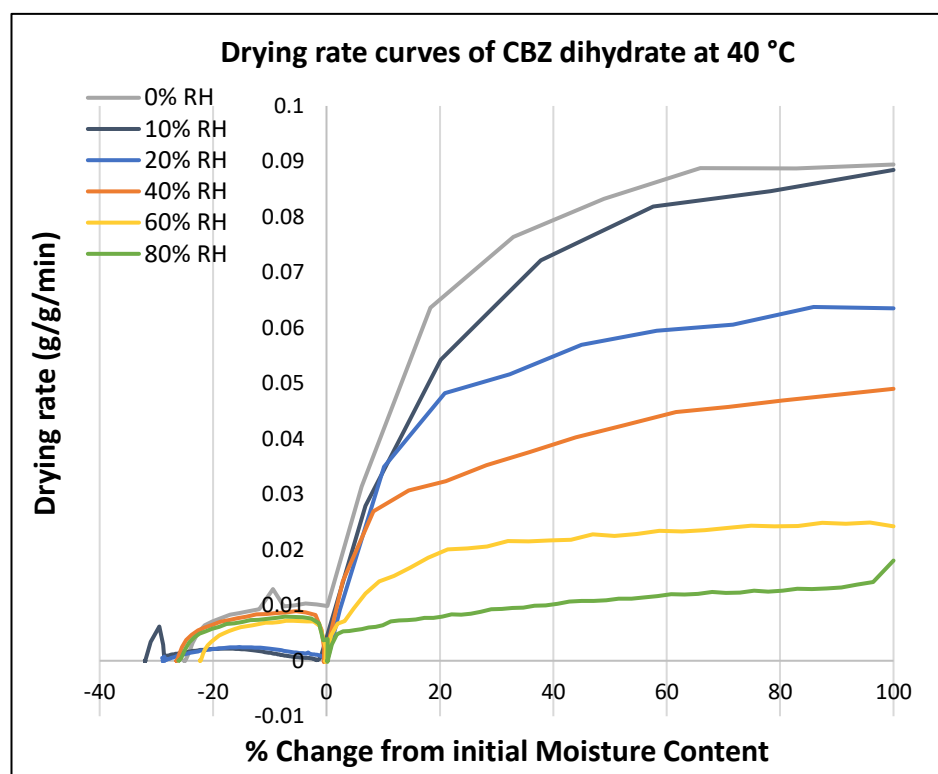


Figure 5.5: Drying rate curves for CBZ dihydrate at 40 °C, at different relative humidities.

5.3.1.1.2. Effects of relative humidity on the hydrate stability of CBZ Dihydrate at 40 °C

The period of hydrate stability is deemed to be where the dry dihydrate sample is present and has 0% excess moisture content.

Figure 5.6 shows a zoomed in portion of the graph shown in **Figure 5.3**. Up to and including a relative humidity of 20% RH, there is minimal hydrate stability. A subtle inflection point (circled on **Figure 5.6**) is seen at 0% RH, as the drying rate changes.

To confirm that the inflection points observed separate regions associated with the removal of different types of moisture (loosely bound moisture and tightly bound water of hydration), a DVS experiment was set up holding a sample at 40 °C and 40% RH for 30 min. The sample was subsequently analysed by TGA.

Figure 5.7 shows the results and confirms the presence of the dry dihydrate. This also proves that the calculation for % M.C in the graphs shown in previous sections is correct, in that values greater than 0% are considered excess, loosely bound moisture.

At 0% RH, all moisture is removed after ~25 min. At 10% RH and 20% RH, there is a small period where, in both cases, dry dihydrate is present. However, the window is short, and dehydration quickly follows. These conditions would not be considered useful or robust drying protocols, as the boundaries denoting dried hydrate and dehydrated hydrate are too close. The hydrate form is stable at $\geq 40\%$ RH, and bound moisture is only removed once induced by the 0%RH step being implemented after 120 min.

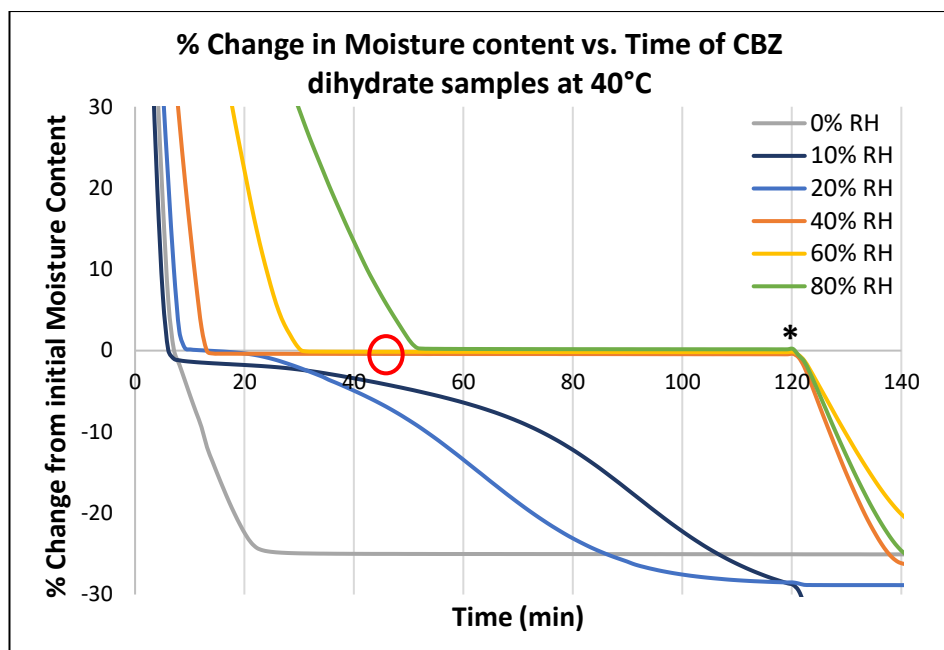


Figure 5.6: % Change in Moisture Content vs. time for CBZ dihydrate at 40 °C and varying %RH, showing the region of hydrate stability, where found. * denotes the start of the 0% RH step.

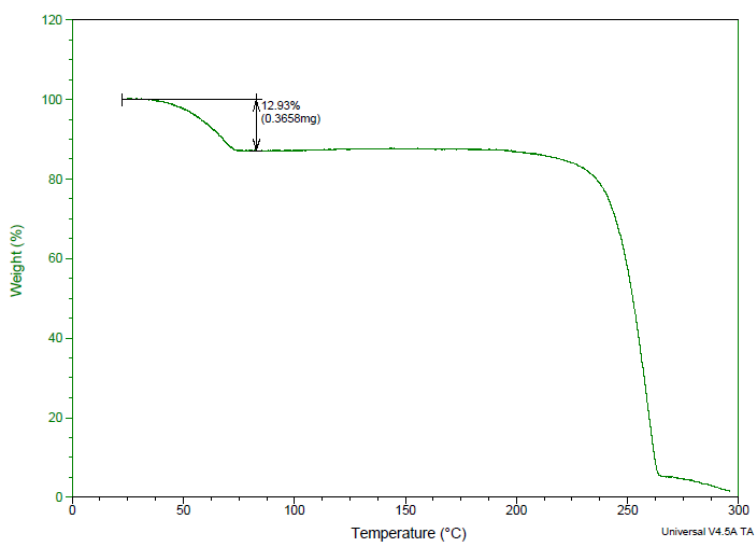


Figure 5.7: TGA Analysis of CBZ dihydrate after being held at 40% RH, 40 °C for 30 min.

5.3.1.1.3. Effects of relative humidity on the dehydration profile after drying of CBZ Dihydrate at 40 °C

If 0% moisture content equates to the dry dihydrate, values <0% relate to the loss of water of hydration and provide the dehydration profile (Region 3, **Figure 5.3**). From **Figure 5.3**, this section is zoomed in on to give a clearer picture of how each sample is affected, see **Figure 5.8**. As previously mentioned, the starting % M.C. for each results set was adjusted to start at 30% to allow for comparison. Due to this, the end points of each system show variation.

Looking at the dehydration curves, as expected, the lowest % RH environment resulted in the fastest loss of moisture, with the end point sample being generated in 25 min. Results for 10% and 20% RH showed dehydration during the initial phase however the presence of a small bump/inflection at 120 min shows that the application of the 0% RH condition (signified by ‘*’) results in the removal of the final amounts of moisture with both samples reaching their end point in 3-5 min thereafter. At 40% RH and above, dehydration only takes place when the 0% RH phase begins, after which, end points were reached approximately 25 min after the step began for the 40% RH experiment, 30 min after for the 60% RH experiment and 33 min after for the 80% RH experiment.

Initial insights into the dehydration at 40 °C shows that the loss of the tightly bound moisture will occur at % RH levels $\leq 20\%$ RH.

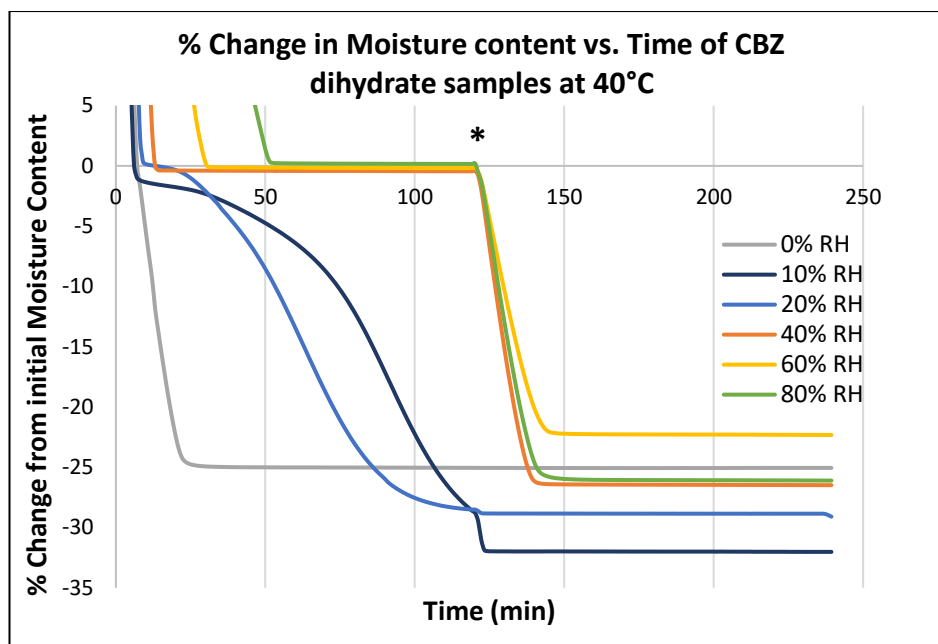


Figure 5.8: % Change in Moisture Content vs. time for CBZ dihydrate at 40 °C and varying %RH, showing the dehydration profiles. * denotes the start of the 0% RH step.

5.3.1.1.4. Solid-state analysis of post DVS end point samples at 40 °C

To investigate the effects of each drying experiment on the CBZ dihydrate crystals, samples were retained post DVS analysis. Due to small sample size, choosing the most beneficial analyses was important. DSC analysis (**Section 2.4.1**) was chosen to confirm the presence or absence of a peak of hydration. SEM (**Section 2.4.8**) gave an insight into changes in the morphology. A newer model XRPD was used (**Section 2.4.5**) to confirm the form present at the end of the DVS run.

5.3.1.1.4.1. DSC Analysis

End point samples did show differences based on drying conditions. **Figure 5.9** shows the DSC overlay of the end point samples after being analysed by DVS at 0, 40, 60 and 80% RH. These thermograms show both the Form III and I melt, as previously discussed, while in **Figure 5.10**, samples that were analysed at 10 and 20% RH show

only the Form I melt, despite the same original sample being used for all experiments. This trend is seen across both the duplicate and triplicate samples. The proposed relationship between CBZ Form III and I and the dihydrate has been discussed in the literature, ^[20] whereby a batch of CBZ dihydrate generated from a Form III polymorph and a batch of CBZ dihydrate generated from a Form I polymorph showed some differences from one another. All the drying study experiments via the DVS in the current study involved a sample of 'dihydrate III', meaning the dihydrate is generated from a suspension of the Form III polymorph in water. The literature details that the application of heat and high humidity on dihydrate III results in Form III being present, while the application of heat and low humidity results in Form I. ^[20] From 40% RH and above, a transformation from dihydrate III to Form III was seen. However, the transformation to Form I for low RH samples was only seen at 10 and 20% RH, with thermograms of those materials (**Figure 5.10**) showing Form I only post DVS. At the lowest level of RH, 0% RH (see **Figure 5.9**), the end point sample showed a small endotherm of the Form III polymorph.

A small endotherm at ~160 °C was seen in the DSC of the 10 and 20% RH samples. This feature is most evident in the 10% RH sample shown in **Figure 5.10**. From the literature a similar endotherm ^[117] but at a lower temperature of 145 °C was discussed as being related to a phase transition from Form III to Form I anhydrate after dehydration of the dihydrate. For the DSC traces shown in **Figure 5.10**, the dihydrate has already been dehydrated through the DVS method used and the higher temperature of the DSC possibly enabled this transition to Form I and could explain the presence of the endotherm at ~160 °C prior to the characteristic melt of the Form I polymorph.

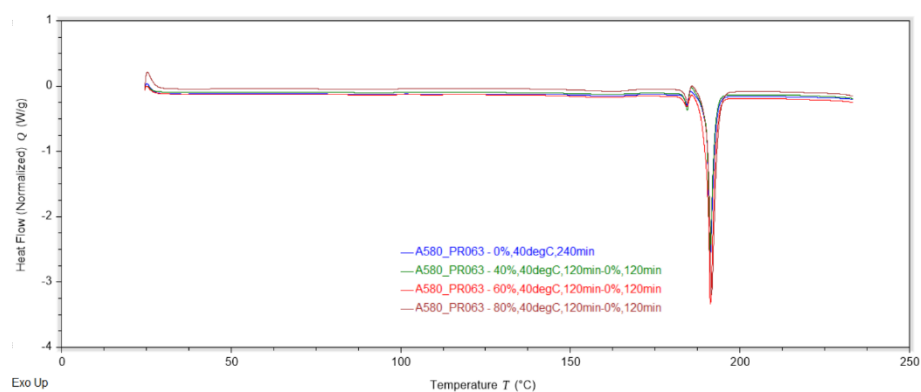


Figure 5.9: DSC overlay of post DVS samples which were analysed at 0, 40, 60, 80% RH.

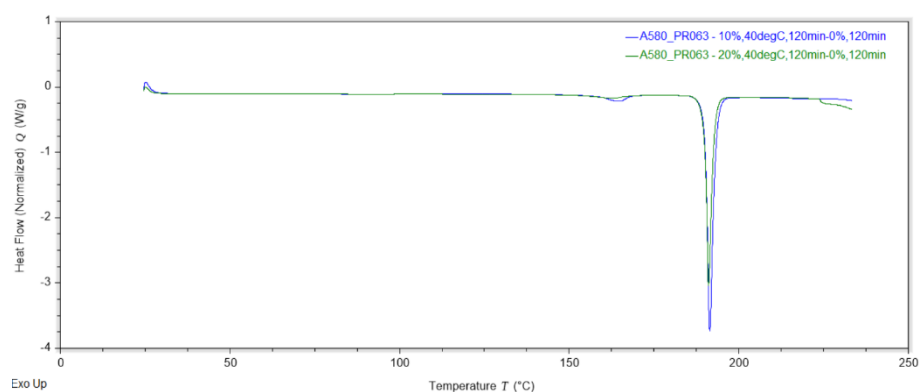


Figure 5.10: DSC overlay of post DVS samples which were analysed in the DVS at 10 and 20% RH.

The enthalpy calculated by the DSC software of each Form I melt, at $\sim 190^{\circ}\text{C}$, in the post DVS samples was lower than that of the same endotherm in the anhydrous starting material and the generated dihydrate. This suggests that under each set of experimental conditions, dehydration resulted in a material which had lower crystallinity than the dihydrate before drying and the form from which the dihydrate was prepared. This change in crystallinity has been previously reported when examining the dehydration of CBZ dihydrate.^[20] It showed that dehydration led to end point samples of lower crystallinity than the form from which they were generated.

5.3.1.1.4.2. SEM Analysis – Gold Sputter Study

For SEM analysis, an initial study was carried out to determine whether gold sputtering was to be used prior to microscopy. For appropriate comparison to be made between the 'before' images of the wet hydrated crystals and the dried 'after' images of the crystals, it was important to both prepare and analyse samples in a way that minimised any additional change to the sample itself. This was most notable for the 'before' images of the wet hydrated forms. These samples contained excess moisture and as vacuum is a factor in both the sputterer and the SEM, minimising exposure where possible was investigated. As discussed in **Section 2.4.8**, for the SEM analysis itself, the lowest level of vacuum was used.

Gold sputtering is used to obtain better, sharper images than is usually achieved without such sputtering, for noncharging surfaces. Using a metal like gold, sputtering increases the conductivity of the samples surface. Without it, an electron charge accumulates, causing a charged effect, resulting in a blurry image.

Figure 5.11 shows images of wet CBZ dihydrate which have been sputtered and **Figure 5.12** shows images of the same wet CBZ dihydrate which has not been sputtered. The surfaces of crystals in both sets of images have similar morphologies and do not look to be affected by the presence or absence of the gold sputtering and then analysis by the SEM. However, the quality of imaging is better when gold sputtering is used. Non sputtered samples (**Figure 5.12**) show extremely white regions which make imaging difficult. CBZ dihydrate is a non-conductive material and as such, can behave as an electron trap, causing electrons to gather on the surface. This is commonly known as charging and these white regions show the evidence of this.

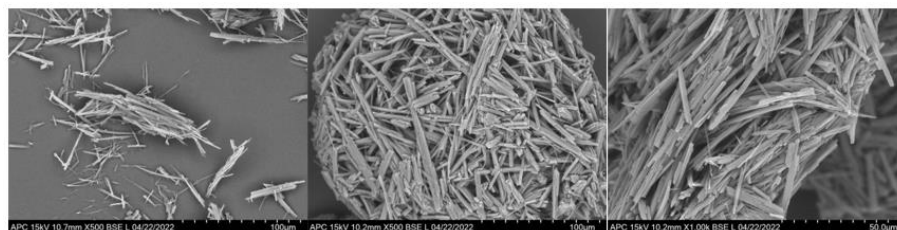


Figure 5.11: SEM images of wet CBZ dihydrate with gold sputtering. x500 (left and middle), and x1k (middle) magnification.

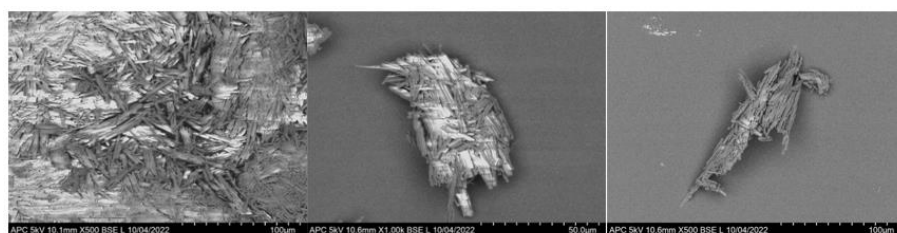


Figure 5.12: SEM images of wet CBZ dihydrate without gold sputtering. x500 (left and right), and x1k (middle) magnification.

Results showed that there were no adverse effects on the samples' morphology caused when using the sputterer. The quality of imaging was affected when gold sputtering was not used. It was decided therefore to continue to sputter both the 'before' and 'after' DVS drying study samples to ensure best quality images could be obtained.

5.3.1.1.5. SEM Analysis

Figure 5.13 shows SEM images of the wet CBZ dihydrate. Images show rodlike crystals with smooth surfaces which have agglomerated together into larger clusters. CBZ dihydrate's characteristic shape, in comparison to the small irregular shape of the anhydrous, is the only shape of crystal present in the images shown below, **Figure 5.13**.

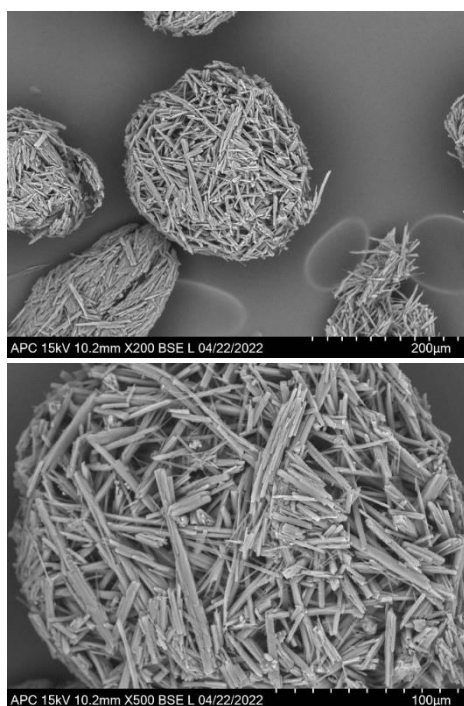


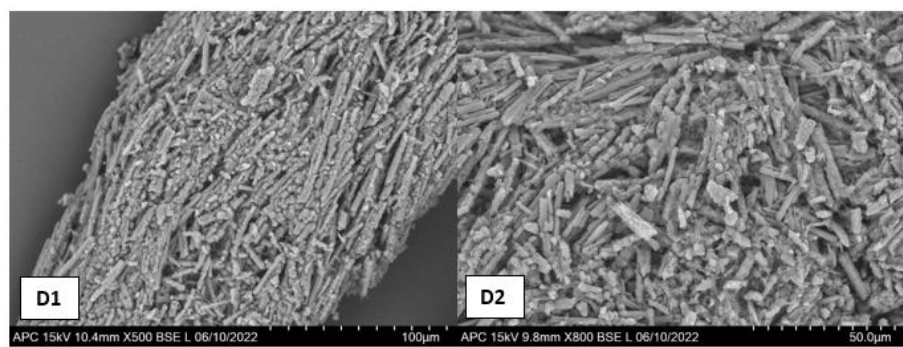
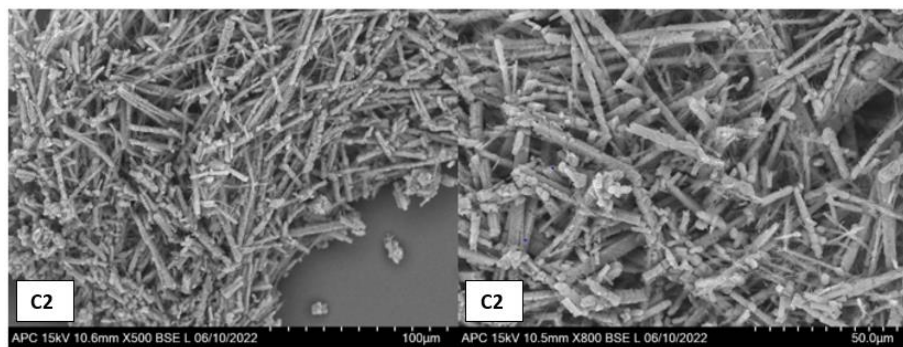
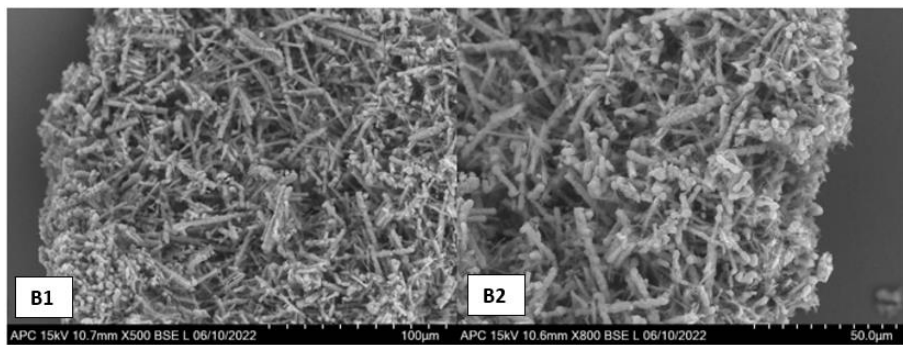
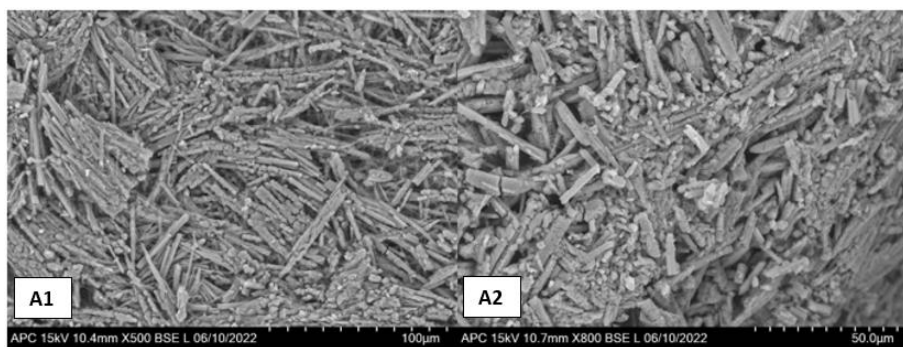
Figure 5.13: SEM images of wet CBZ dihydrate prior to drying studies via DVS.

Figure 5.14 shows the SEM images of post DVS drying study samples after being analysed at 40 °C and at increasing % RH levels. Across all experimental conditions, the post DVS samples look to have similar morphological changes to one another. Crystal surfaces are no longer smooth as shown in **Figure 5.14** but show ridges and uneven surfaces. Previously smooth, rodlike hydrated crystals now have ridges which track linearly, supporting the presence of channels in CBZ dihydrate, with ridges forming as a result of water being removed from the channels. Also, in these post DVS images, the presence of very small irregular shaped particles (see **Figure 5.14 , D1**) is evident, suggesting the presence of the CBZ anhydrous form. More interestingly, using the x800 magnification, the presence of very fine whisker-like crystals is seen, (**Figure 5.15**).

Examining these fibres more closely, **Figure 5.15** shows that after drying and dehydration, these whiskers are growing from the

previously hydrated crystals and stretching outwards. This fibre growth after dehydration has been discussed in the literature in relation to solvent-induced transformations of hydrated compounds. ^[122] When dehydrated using an organic solvent, high local supersaturations during the phase change forces dendritic growth to take place where the stable form, after transformation, also has a very low solubility in the solvent. ^[122] While organic solvents are not used in these DVS drying studies, considering the highly controlled nature with which the samples are being dried/dehydrated, the equilibrium concentration of the sample and vapour may be causing changes to the supersaturation levels on the surface of the hydrated crystals resulting in the dendritic growth of anhydrous whiskers.

In initial work on CBZ dihydrate generation, **Section 2.3.1.2**, similar fibres were seen and were discussed in relation to the whisker theory of growth. From the literature ^[106], the whiskers are considered to be the starting point of the dihydrate and they were considered dihydrate whiskers themselves. However, post DVS, in **Section 5.3.1.1.4.1**, no peak of hydration is seen in any of the DSC thermograms. This suggests that these whiskers are not of the dihydrate.



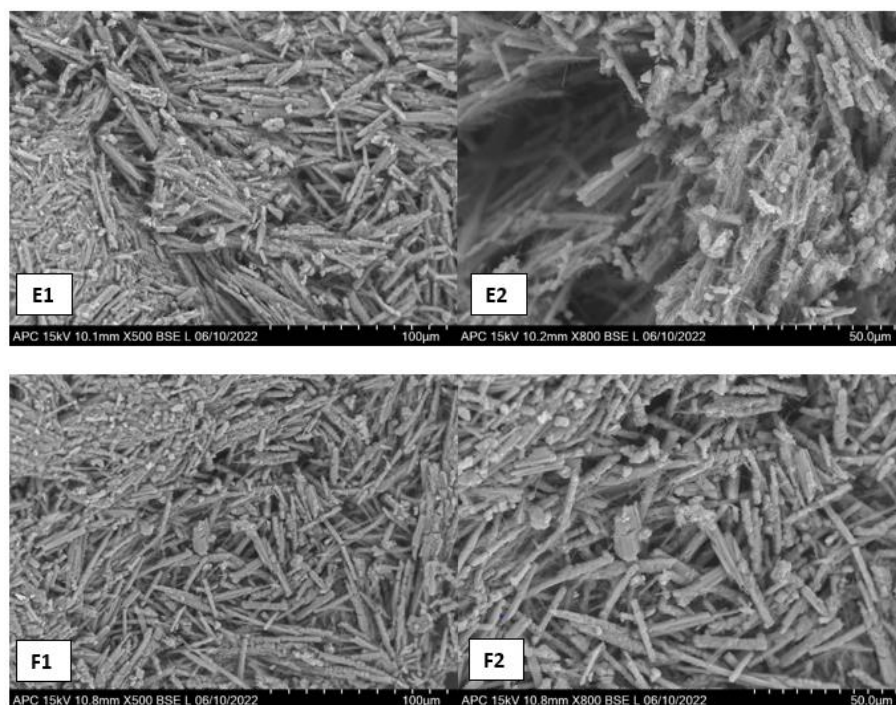


Figure 5.14: SEM images of Post 40 °C DVS drying studies of CBZ dihydrate at **A1)** 0% RH, x500, **A2)** 0% RH, x800, **B1)** 10% RH, x500, **B2)** 10% RH, x800, **C1)** 20% RH, x500, **C2)** 20% RH, x800, **D1)** 40% RH, x500, **D2)** 40% RH, x800, **E1)** 60% RH, x500, **E2)** 60% RH, x800, **F1)** 80% RH, x500, **F2)** 80% RH, x800.

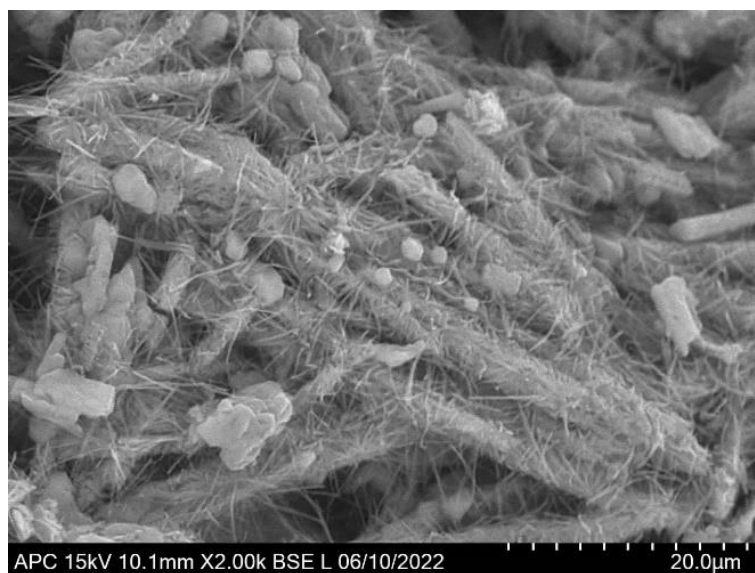


Figure 5.15: SEM image of post 40 °C DVS drying of CBZ at 60% RH at x2000 magnification.

5.3.1.1.5.1. XRPD Analysis

While sample size does not affect the resolution achievable, the newer instrument used in the work of the current chapter allowed for analysis of samples with only ~ 10-20 mg. To aid end point comparisons, portions of the CBZ dihydrate pre-DVS drying study and the anhydrous starting material were also analysed with the newer instrument. **Figure 5.16** shows an overlay of the anhydrous CBZ starting material and a portion of the batch of 'wet' dihydrate from which all samples analysed by DVS were taken. Clear differences are seen between the samples, and this will aid comparison of the end point samples, post DVS.

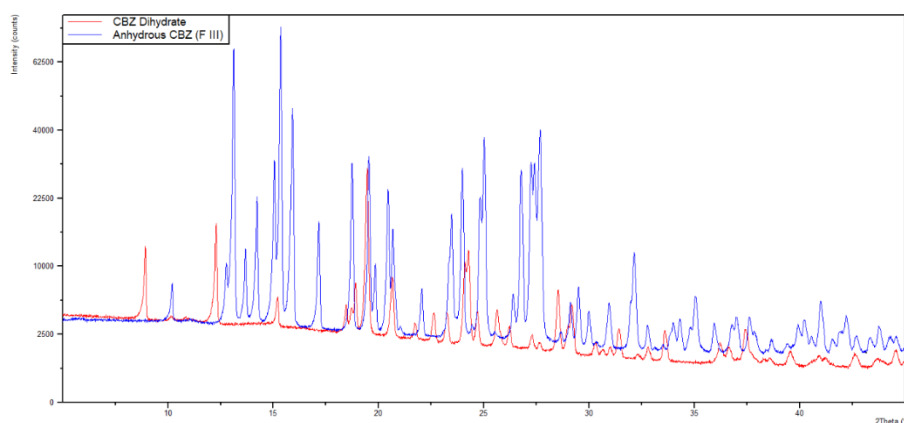


Figure 5.16: XRPD overlay of the 'wet' CBZ dihydrate (red) prior to the DVS drying studies and the anhydrous CBZ Form III starting material (blue).

Like the DSC analysis of the same samples, differences in post DVS samples were seen depending on the experimental conditions. Traces of samples analysed at 0, 40, 60 and 80% RH at 40 °C, **Figure 5.17**, overlaid with one another. There were no common peaks with the pre-drying study, 'wet' dihydrate, and while most peaks corresponded to peaks in the anhydrous starting material (Form III), there were two additional peaks present which could not be accounted for. These peaks were found at ~16.5° 2θ and ~18° 2θ .

As sample amount was limited, so is the resolution of each XRPD pattern, making comparisons and peak identification more difficult. The peak at $\sim 16.5^\circ 2\theta$ is smaller in comparison to the one at $\sim 18^\circ 2\theta$. From the literature, a peak for CBZ around $\sim 18^\circ 2\theta$ could relate to the anhydrous Form II polymorph, due to preferred orientation of the crystals. [78] However, when coupled with DSC data, this cannot be concluded. As previous indicated, DSC thermograms of these samples show the clear presence of the Form III polymorph which melts, recrystallises to the Form I polymorph, followed by its own characteristic melt. The presence of Form III after dehydration of CBZ dihydrate at a wide range of % RH levels has previously been seen. [75]

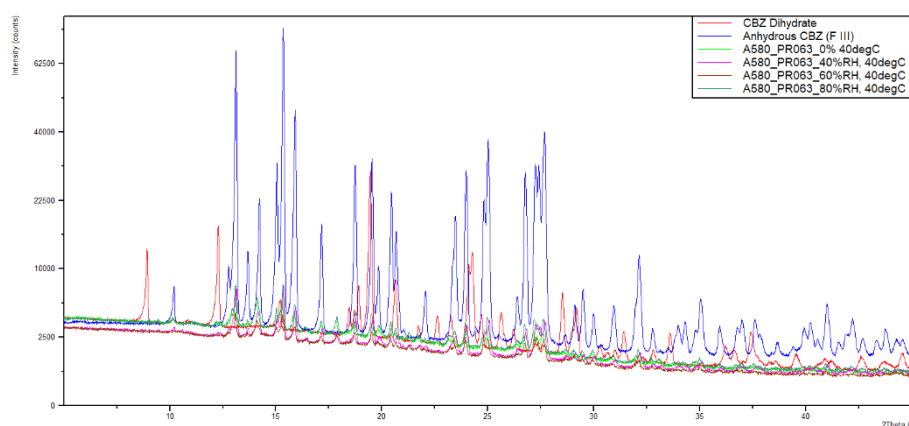


Figure 5.17: XRPD overlay of ‘wet’ CBZ dihydrate (red), anhydrous CBZ Form III starting material (blue), and post DVS samples after analysis at 0 (green), 40 (pink), 60 (brown) and 80% RH (dark green) at 40 °C.

Post DVS samples analysed at 10 and 20% RH at 40 °C, both shown in **Figure 5.18**, overlay with the anhydrous CBZ starting material with no additional or lost peaks. When compared with the ‘wet’ dihydrate pattern, there are no common peaks. This was unexpected since, as shown in **Figure 5.10**, the DSC traces of these samples did not show the presence of the Form III melt, only that of the Form I melt. A possible explanation for this is that, as previously mentioned in **Section 5.3.1.1.4.1**, post DVS, the

dihydrate sample has been dehydrated and this resulted in disordered CBZ crystals, which is the sample being analysed. When heated to high temperatures in the DSC (**Figure 5.10**), there is a small baseline disturbance which is attributed to a Form III to Form I transition, whereas the XRPD does not reach those temperatures for this transition to take place.

At these lower humidity conditions, the samples are believed to transform from the dihydrate to the Form III polymorph with CBZ crystals having some disorder due to dehydration from the DVS method.

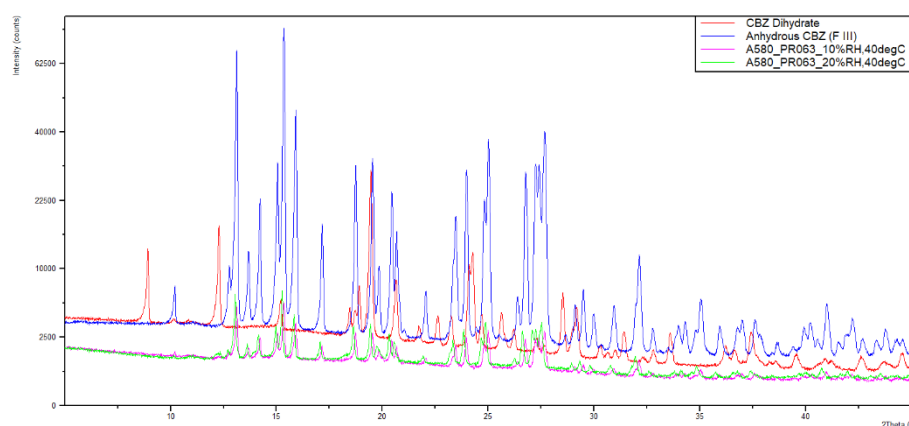


Figure 5.18: XRPD overlay of ‘wet’ CBZ dihydrate (red), anhydrous CBZ Form III starting material (blue), and post DVS samples after analysis at 10 (pink) and 20% RH (green) at 40 °C.

5.3.1.1.5.1.1. Non-ambient XRPD Analysis

A non-ambient XRPD chamber (**Section 2.4.5.1**) was used to mimic DVS experiments on the same sample of CBZ dihydrate to track the changes via its XRPD trace while also confirming the form of the end point sample. Due to scan lengths taking 6-8 min (fastest option available), each trace does not correspond to each DVS data point, however, this analysis could provide an insight into what is occurring mid DVS experiment. While the number of data points is less than the DVS, a large number of traces are collected over each

4 hr period and, to examine these better, only every second trace is graphed in the next three sections, unless a significant difference was evident. Also, where a change in conditions was required, e.g., moving from 40 % RH to 4% RH, there is a short transition time where this takes place. Only after this and when the new conditions are stable does the next scan begin. So, while extra information is gathered here, it cannot be directly compared to that of the DVS. Finally, 0% RH was not achievable on this instrument; 4% RH was lowest value that could be obtained so this was used instead to compare with the 0% RH condition in other experiments.

5.3.1.1.5.1.1.1. 4% RH, 40 °C for 4 hr

Shown in **Figure 5.19** are the consecutive XRPD scans of the drying and dehydration of 'wet' CBZ dihydrate at 4% RH, 40 °C over 4 hr. Moving through the scans the gradual disappearance of the characteristic peaks of the dihydrate was followed by the appearance of a crystalline anhydrate phase. The initial broad peak seen at $5.5^\circ 2\theta$ is due to the instrument specific holder used and is not related to the CBZ sample.

Figure 5.20 confirmed the presence of the dihydrate at the start of the experiment. After 4 hr, the final scan appears to show a mixture of forms (**Figure 5.21**). This mixture of forms during analysis with ethanol vapour has previously been discussed ^[83] and was suspected to be due a crystalline intermediate which then converted to a mixture of anhydrous Form III (starting material) and anhydrous Form II. The presence of new peaks at $<10^\circ 2\theta$ in **Figure 5.21** confirms the same type of mixture of forms in the current study. There is one peak in the final scan at $\sim 12.4^\circ 2\theta$ which corresponds to a peak in the dihydrate trace only. The intensity of the peak is greatly reduced relative to the same peak in the first scan, suggesting the transition from the dihydrate is not fully

completed. The previously mentioned article ^[83] also presented the effects of increasing % RH levels between 0% RH and 5% RH on the dehydration of CBZ dihydrate ^[83], and showed how even small changes in RH had an impact on the crystallinity of the end point sample. An increase in water vapour pressure saw an increase in the crystallinity in the anhydrate form. This is worth noting for the comparison of these end point samples and the post DVS XRPD results in **Section 5.3.1.1.5.1**, as those samples were held in the highly controlled environment of the DVS which could achieve a lower level of % RH.

Coupled with DVS work in **Sections 5.3.1.1.1** and **5.3.1.1.3**, the lack of stability of the dihydrate form at similar conditions (0%/4% RH) is confirmed again through these XRPD results.

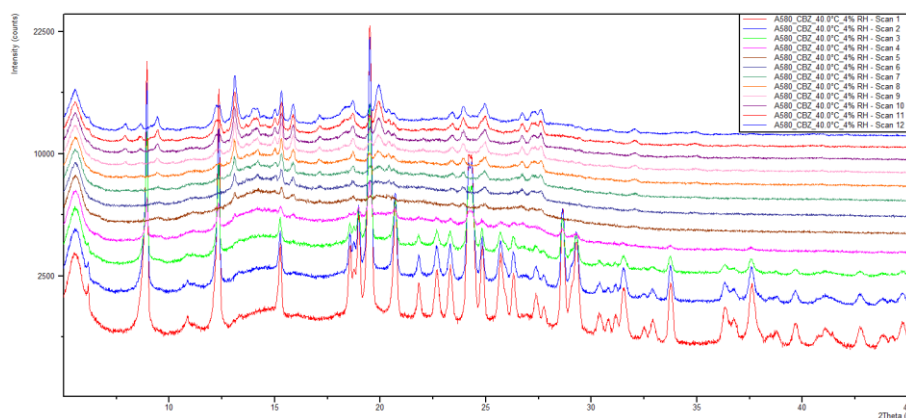


Figure 5.19: Non-ambient XRPD overlay of CBZ dihydrate being held at 4% RH, 40 °C for 4 hr. (Bottom to top - first scan to last scan).

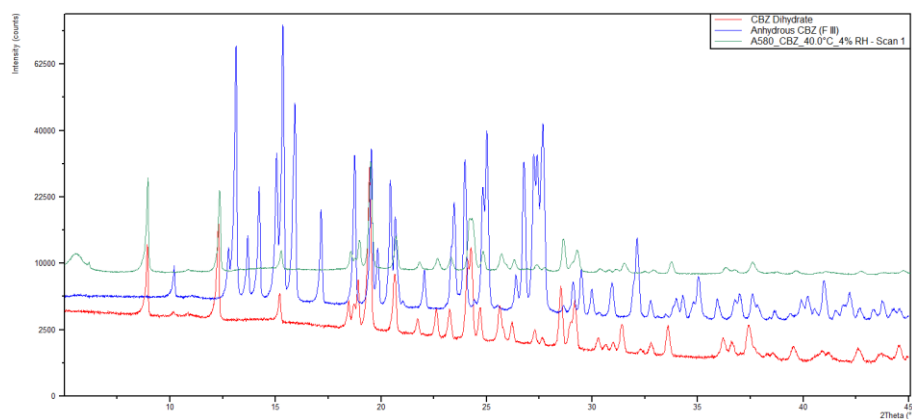


Figure 5.20: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the first scan of CBZ dihydrate being held at 4% RH, 40 °C.

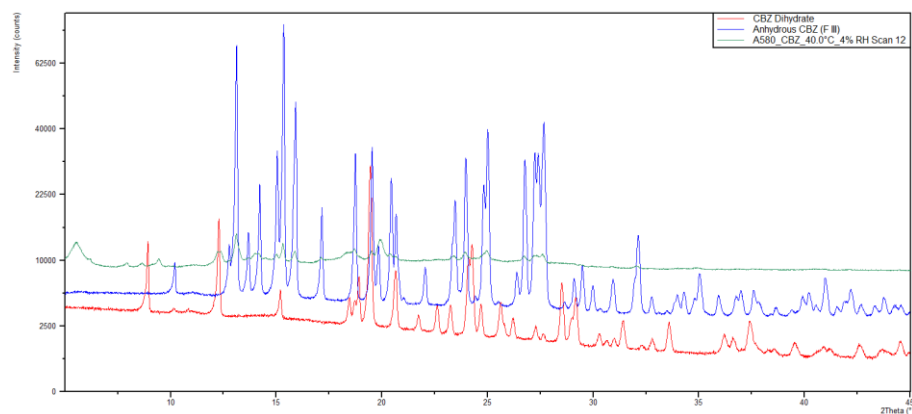


Figure 5.21: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the last scan of CBZ dihydrate after being held at 4% RH, 40 °C for 4 hr.

5.3.1.1.5.1.1.2. 40% RH, 40 °C for 2 hrs, switching to 4% RH, 40 °C for 2 hr

Figure 5.22 shows the XRPD overlay of scans throughout the experiment, with the change in % RH (from 40 to 4% RH) made clear through the extra space after the first four scans. The gradual disappearance of the characteristic peaks of the dihydrate was only evident after the % RH value was reduced to 4% RH. **Figure 5.23** confirmed the presence of the dihydrate at the start of the experiment. All scans at 40% RH showed the dihydrate was present. The first scan at 4% RH showed many of the characteristic peaks of

the dihydrate but with a decrease in intensity. From the second scan at 4% RH, the appearance of anhydrous peaks is clear. The final scan, **Figure 5.24**, showed a similar trace to that shown in **Figure 5.21**. The presence of new peaks at $<10^\circ 2\theta$ was seen again, along with the singular dihydrate peak at $12.4^\circ 2\theta$.

Coupling this work to the similar experiment carried out in the DVS, reported in **Sections 5.3.1.1.1** and **5.3.1.1.3**, provides confirmation that the dihydrate can remain stable at 40% RH and 40 °C, after drying off excess moisture, until the lower % RH step is implemented.

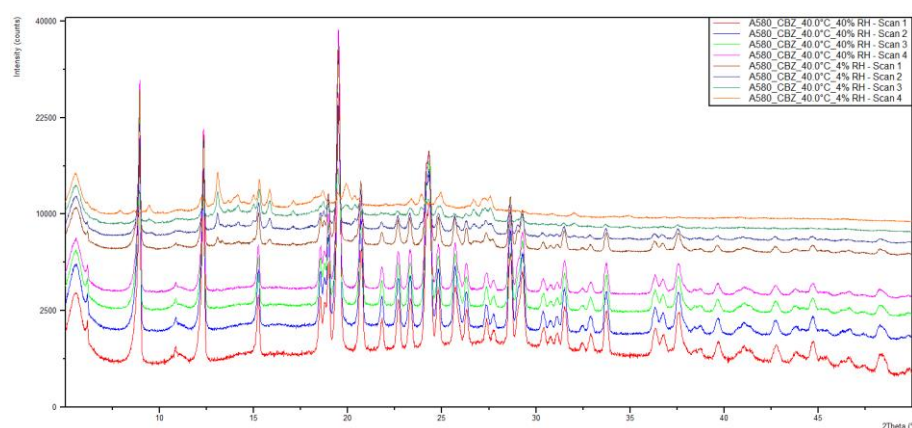


Figure 5.22: Non-ambient XRPD overlay of CBZ dihydrate being held at 40% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr. (Bottom to top - first scan to last scan).

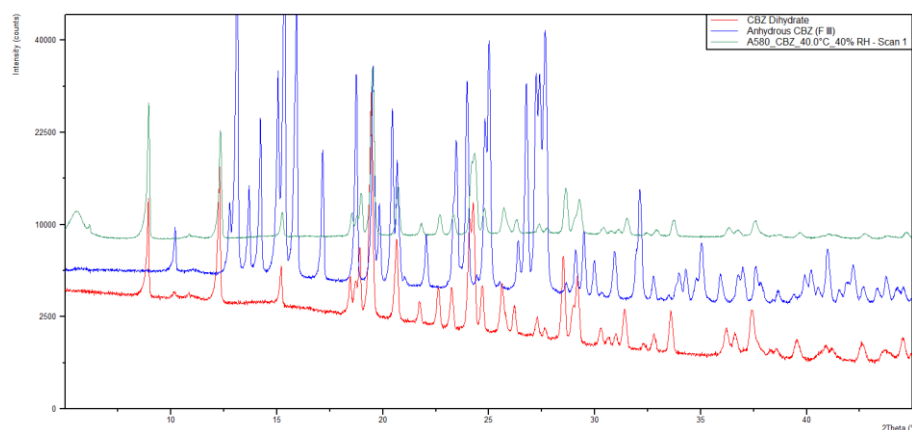


Figure 5.23: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the first scan of CBZ dihydrate being held at 40% RH, 40 °C.

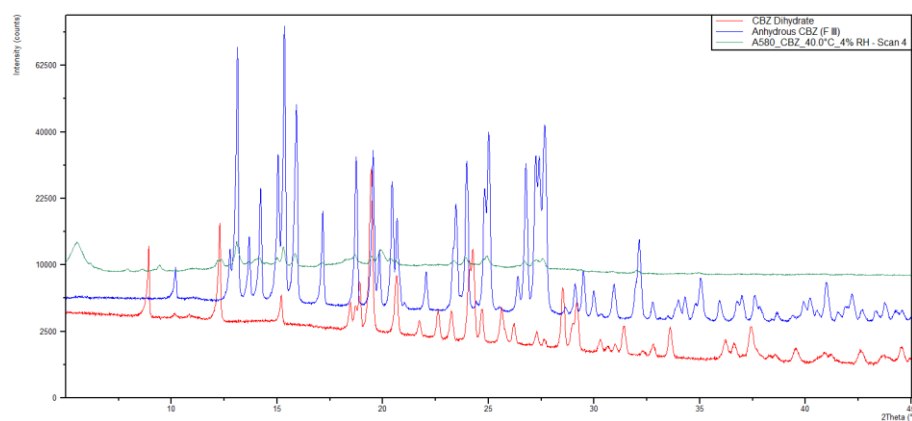


Figure 5.24: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the last scan of CBZ dihydrate after being held at 40% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr.

5.3.1.1.5.1.1.3. 80% RH, 40 °C for 2 hrs, switching to 4% RH, 40 °C for 2 hr

Figure 5.25 shows the XRPD overlay of the full experiment, with the change in % RH (from 80 to 4% RH) made clear through the extra space after the first four scans. Based on conclusions from DVS work in **Sections 5.3.1.1.1** and **5.3.1.1.3**, the loss of the characteristic peaks of the dihydrate was only seen once the % RH value was reduced to 4% RH. **Figure 5.26** confirmed the presence of the dihydrate at the start of the experiment. From the second

scan at 4% RH, the trace of a largely amorphous phase is seen. This transition to an amorphous form has been discussed in the literature and is related to the breaking of the amide C=O bond, as part of the first process of dehydration within CBZ dihydrate. ^[118] By the final scan, a small number of poorly resolved crystalline peaks can be seen. This final scan, **Figure 5.27**, is poorly resolved when compared to the dihydrate and starting material traces. However when overlayed with end point sample traces from **Sections 5.3.1.1.5.1.1.1** and **5.3.1.1.5.1.1.2**, despite it being poorly resolved, similarities can be seen, especially to the anhydrous CBZ Form III trace. One clear difference is the absence of the peak at $12.4^\circ 2\theta$. This suggests that during dehydration, after being held at 80% RH, CBZ forms amorphous material initially and a final semi-crystalline form, with the amorphous material re-ordering itself and giving more of the final form over time with no evidence of the previous dihydrate form.

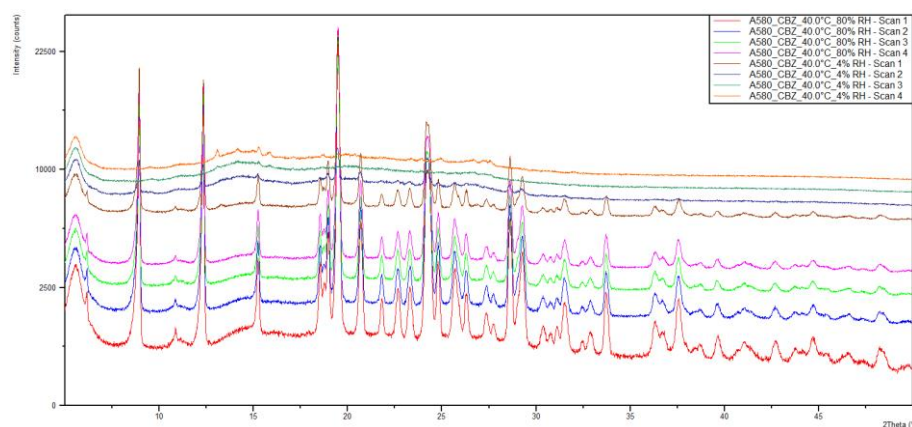


Figure 5.25: Non-ambient XRPD overlay of CBZ dihydrate being held at 80% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr. (Bottom to top - first scan to last scan).

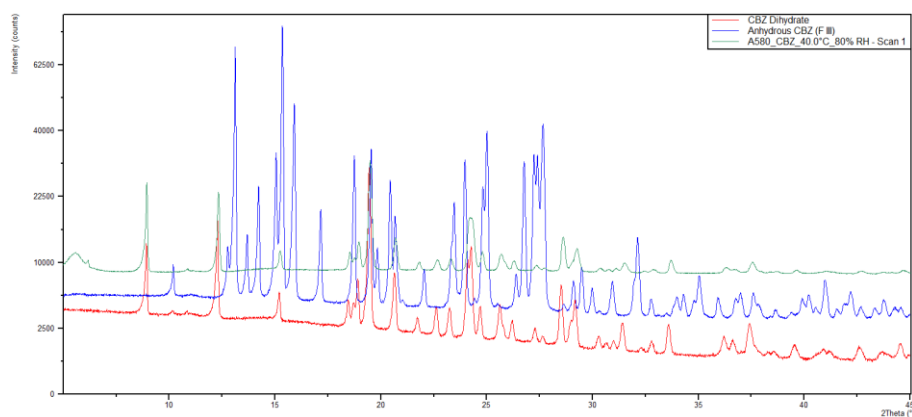


Figure 5.26: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the first scan of CBZ dihydrate being held at 80% RH, 40 °C.

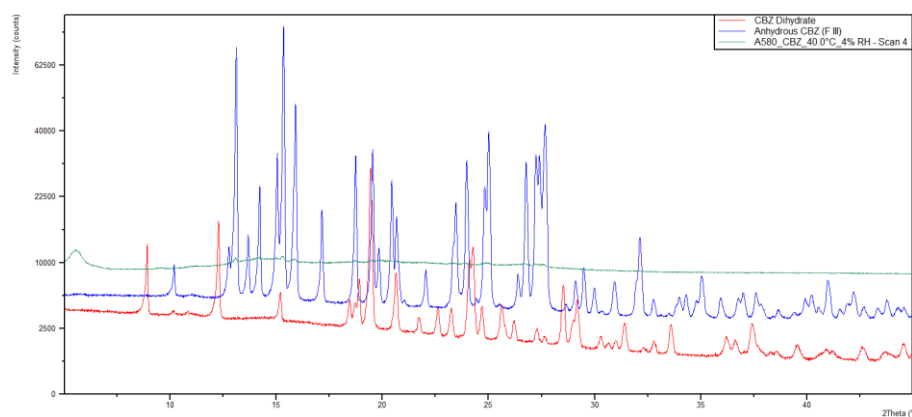


Figure 5.27: Non-ambient XRPD overlay of CBZ dihydrate, anhydrous CBZ Form III and the last scan of CBZ dihydrate after being held at 80% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr.

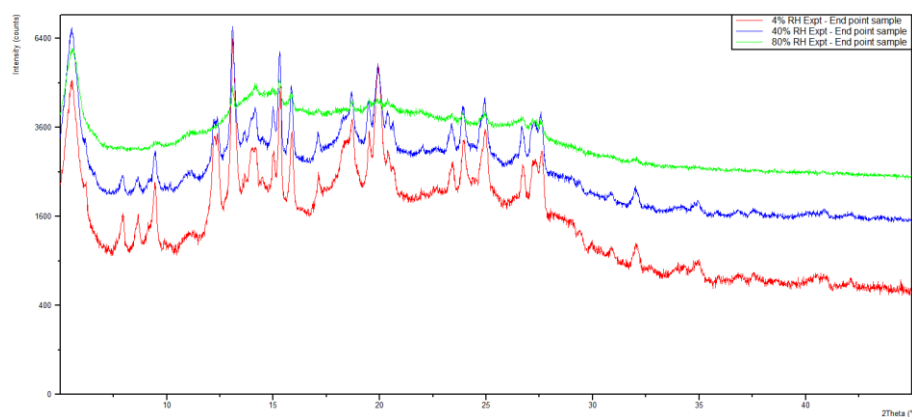


Figure 5.28: Non-ambient (40 °C) XRPD overlay of end point samples for CBZ at different % RH (4% RH, 40% RH and 80% RH, from bottom to top).

5.3.1.2. Effects of temperature on the drying of wet CBZ dihydrate at 30 °C and 40 °C

To investigate the effects of temperature, portions of the same CBZ dihydrate sample (as were analysed in the DVS at 40 °C) were analysed at 30 °C at the same % relative humidities of 0, 10, 20, 40, 60 and 80% RH.

Figure 5.29 shows the full profiles of each sample analysed at 30 °C and 40 °C. As mentioned in relation to **Figure 5.3**, the drying profiles (loosely bound moisture) (1), period of hydrate stability (2), where conditions allowed, and the dehydration profiles (3) are detailed on the graph, along with the start of the 0% RH step (signified by the '*').

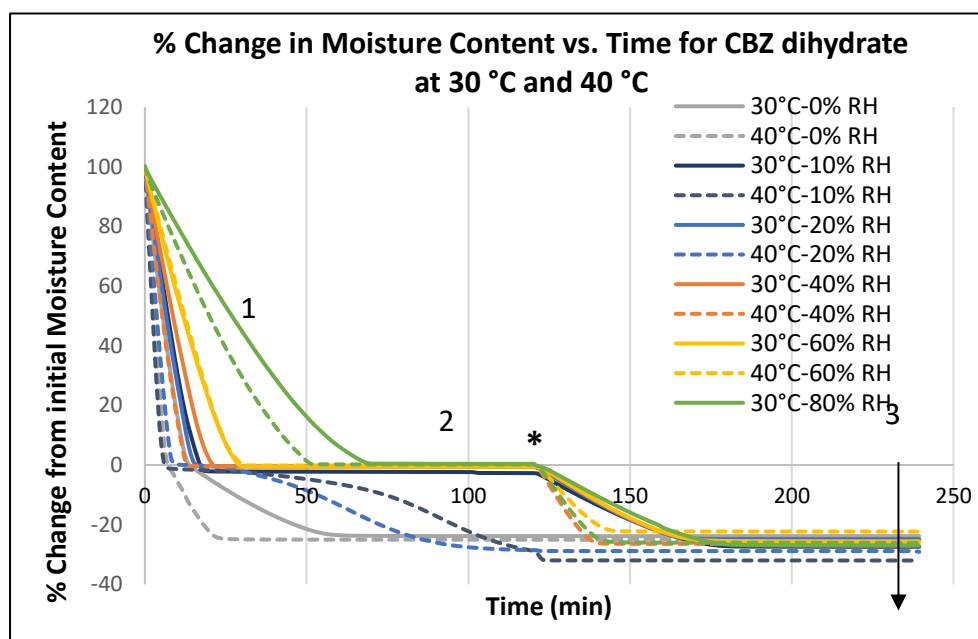


Figure 5.29: % Change in Moisture Content vs. time for CBZ dihydrate samples at 30 °C and 40 °C, with % relative humidities ranging from 0-80% RH. * denotes the start of the 0% RH step.

5.3.1.2.1. Effects of temperature on the drying profile of CBZ Dihydrate

To examine the drying profile, **Figure 5.30** shows a zoomed in section from the original profiles shown in **Figure 5.29**.

Overall, the trends showed that a higher temperature gave shorter drying times except in the case of 60% RH, where an increase in temperature of 30 °C to 40 °C did not affect the drying time. Triplicate analysis of these samples confirmed this interesting finding (see **Figure 5.31**). To investigate whether drying at high levels of RH still provides an environment for hydrate stability at higher temperatures, this set of experiments could be repeated at another higher temperature point. The DVS Intrinsic used in this study has an operational temperature range of 20-40 °C so this is not possible currently but could be completed with a different model of DVS. Drying times, across all runs is shown in **Table 5.2**. Drying times ranged from less than 7 min to 70 min.

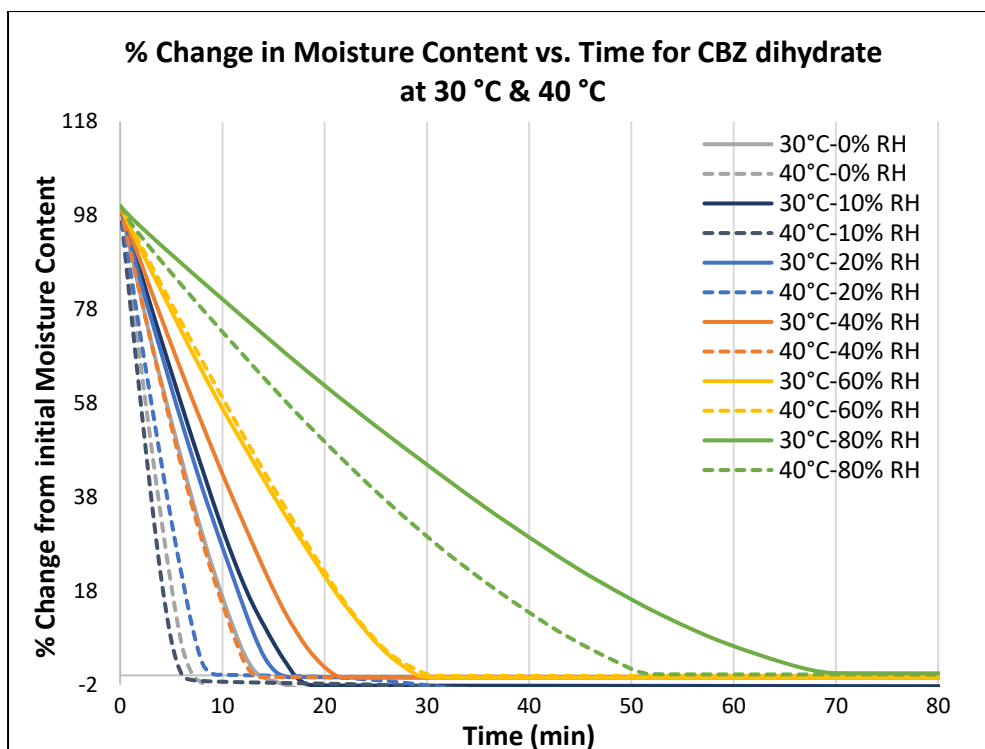


Figure 5.30: % Change in Moisture Content vs. time for CBZ dihydrate samples at 30 °C and 40 °C and varying %RH, showing the drying profiles.

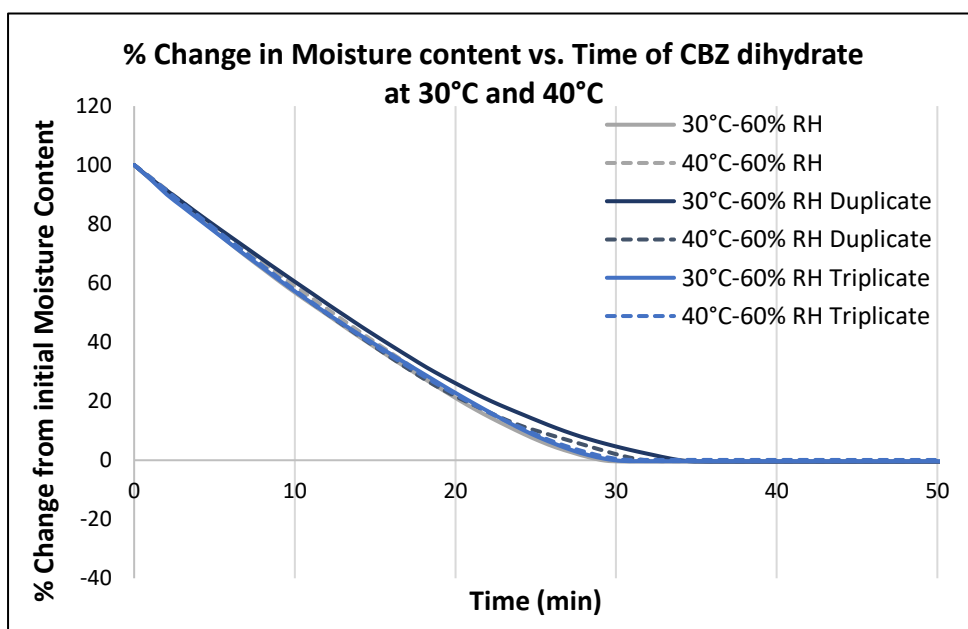


Figure 5.31: % Change in Moisture Content vs. time for CBZ dihydrates samples dried at 60% RH, in triplicate, at 30 °C and 40 °C, showing the drying profiles.

Table 5.2: Drying times of samples at increasing % RH environments at both 30 °C and 40 °C.

% RH	30 °C ⁽¹⁾	30 °C ⁽²⁾	30 °C ⁽³⁾	40 °C ⁽¹⁾	40 °C ⁽²⁾	40 °C ⁽³⁾
	<i>Drying time (min) – to arrive at dry hydrate</i>					
0	14	11	14	8	9	9
10	18	14	14	7	8	10
20	16	11	16	10	9	12
40	22	20	30	14	14	12
60	30	34	30	30	31	30
80	70	52	52	52	52	43

Examining the traditional drying rate curves shown in **Figure 5.32**, higher temperatures gave an increase in drying rates across all samples. The differences in the rate of drying caused by the differing temperatures, was the greatest in the 10% RH samples. The difference reduced with increases to the % RH environments. Examining this graph further and coupling it with experiment discussed at the end of **Section 5.3.1.1.2** , by reaching the origin and having 0% M.C. and a drying rate of 0, it can be deduced that a period of hydrate stability (i.e. a period of time where the dry dihydrate is present) takes place. The 0% RH samples is the only sample with no period of hydrate stability.

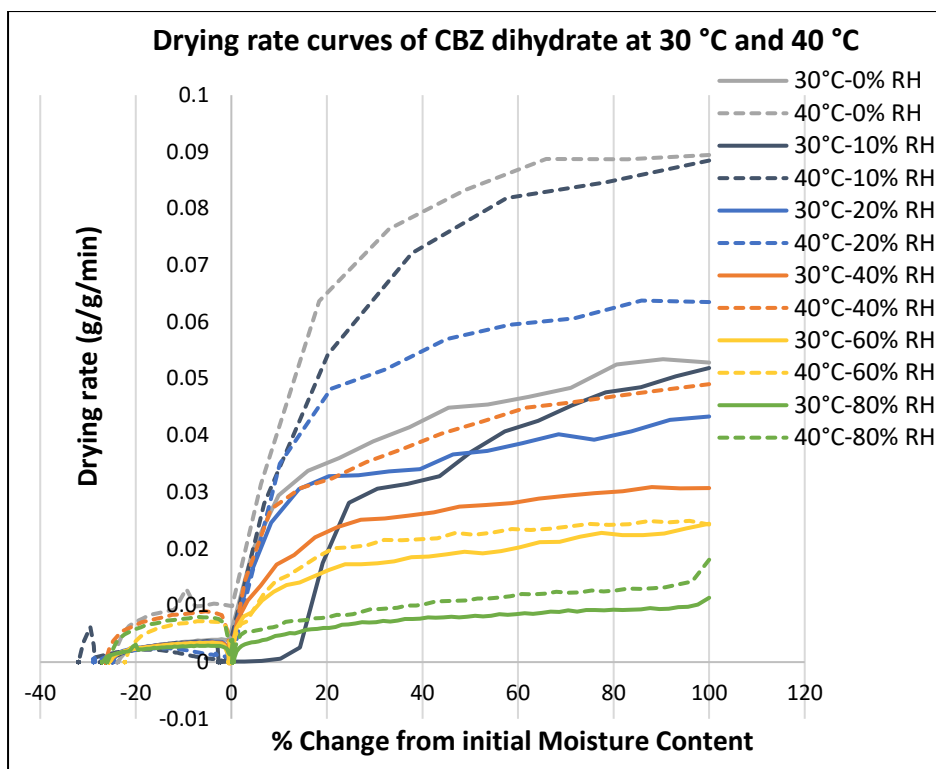


Figure 5.32: Drying rate curves for CBZ dihydrate at 30 °C and 40 °C, at different relative humidities.

5.3.1.2.2. Effects of temperature on hydrate stability of CBZ dihydrate

From both samples (30 °C and 40 °C) at 0% RH are the only ones that show no period of hydrate stability. For these samples, an inflection point at 0% M.C. is the only indication of change, see **Figure 5.33**. The experiment discussed in **Section 5.3.1.1.2** confirms that this inflection point signifies the shift from the removal of loosely bound moisture to the removal of tightly bound, water of hydration.

Looking at samples analysed at 30 °C, hydrate stability is seen across all samples at 10% RH and above. As is clearly visible in the graph, the straight-line relating to hydrate stability is sitting below the 0% M.C. for the 10% RH sample. This was seen across triplicate analyses of this sample. Despite this, a stable % M.C. was found

after drying (i.e., Region 1) up until the 120 min time point. The extent of the stability would suggest the presence of the dry dihydrate.

With the temperature increase, extended periods of hydrate stability were not seen until the % RH increased to 40% RH. Below this, at 10% RH, while there is a point of inflection, and a definite change in slope, there is still no obvious period of hydrate stability at these conditions. At 20% RH, hydrate stability is seen for only very short period of ~ 5 min. It is evident that these conditions would be unsuitable for drying CBZ dihydrate to consistently produce the dry dihydrate.

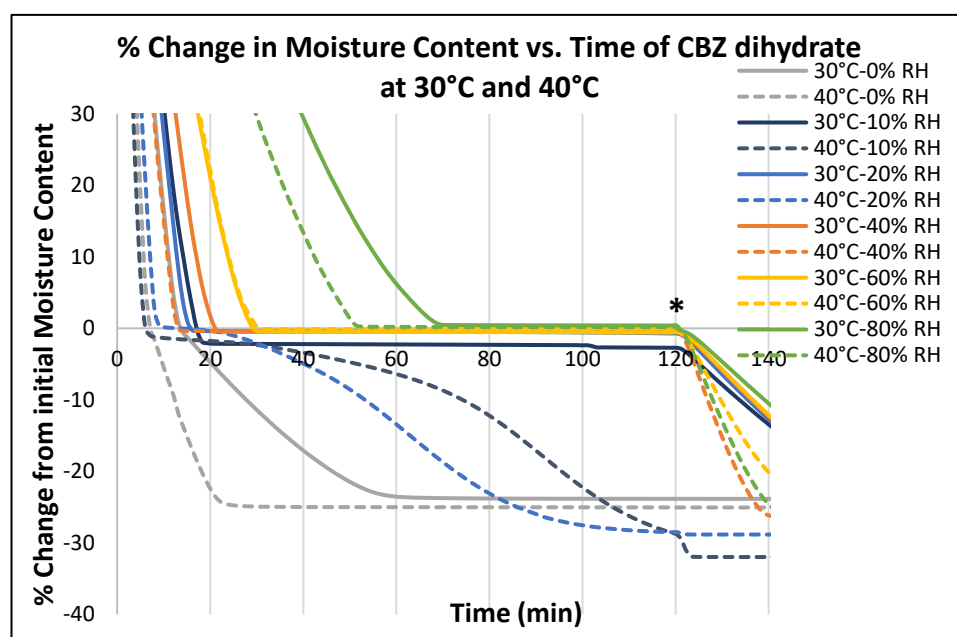


Figure 5.33: % Change in Moisture Content vs. time for CBZ dihydrate samples at 30 °C and 40 °C and varying %RH, showing periods of hydrate stability (when % moisture content equals 0%). * denotes where the 0% RH step commences.

5.3.1.2.3. Effects of temperature on the dehydration profile of CBZ Dihydrate

Overall, a higher temperature resulted in less time taken to remove the water of hydration. Also, regardless of the % RH environment being low or dehydration being induced by the 0% RH step, dehydration time remained consistent at each temperature: 50-60 min for samples at 30 °C and 20-30 min for samples at 40 °C.

As mentioned in the previous section, 0% RH samples analysed at 30 °C and 40 °C moved from a phase of drying off loosely bound moisture straight into the dehydration of the remaining moisture. The start of dehydration was denoted by a change in slope with a point of inflection. Once the relative humidity was increased, samples at 30 °C consistently dehydrated only once the 0% RH step began after 120 minutes (see **Figure 5.34**), after which, dehydration consistently took between 50-60 minutes across all % RH environments. At 40 °C, samples were more sensitive to the differences in % RH. During the 10% RH experiment, with minimal hydrate stability seen, dehydration took place during the 10% RH step. Dehydration continued up to the 120 min mark, at which point there was still a small amount of moisture remaining. Once the 0% RH step began, the remaining moisture was quickly removed within 5 min and is graphically shown by the sudden decrease in % M.C. of the sample. Similarly at 20% RH, after a short-lived period of hydrate stability, dehydration began during the 20% RH step, starting at ~16min and showing a gradual loss of moisture up to 120 min. When the 0% RH step began, the small amount of remaining moisture was also quickly removed. At 40% RH and above, dehydration did not begin until induced by the start of the 0% RH, with the water of hydration being removed within 20-30 min thereafter.

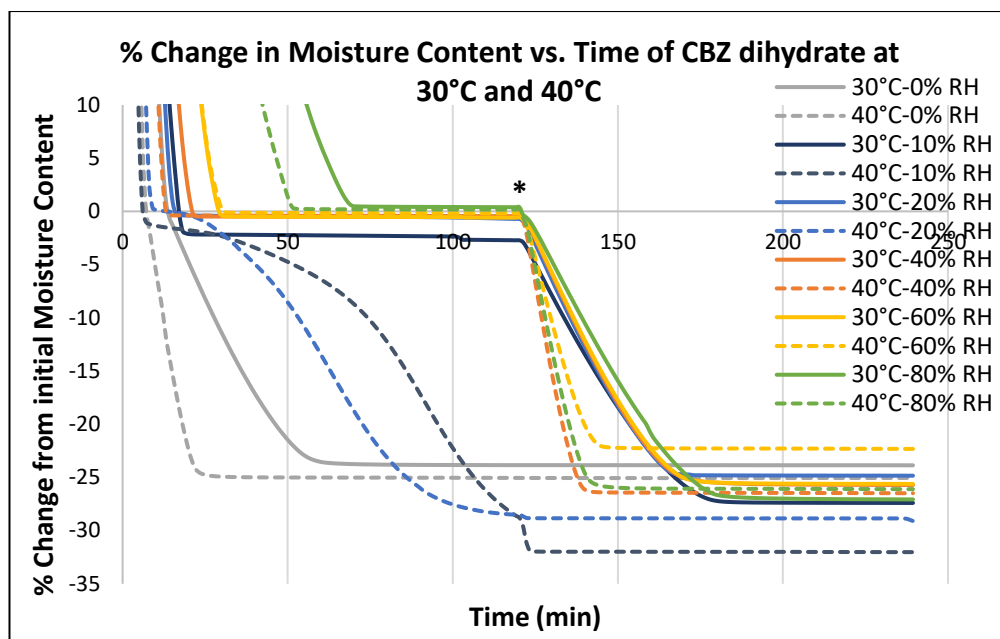


Figure 5.34: % Change in Moisture Content vs. time for CBZ dihydrate samples at 30°C and 40°C and varying %RH, showing the dehydration profiles. * denotes where the 0% RH step commences.

5.3.1.3. Effects of particle size on the drying of wet CBZ Dihydrate at 40 °C

To investigate the effects of particle size on the drying, stability, and dehydration of CBZ dihydrate, portions of ‘wet’ CBZ dihydrate were generated of increasing particle size. The samples discussed here are different to those of differing PSD discussed in **Section 3.3.4.2**, whether by generating new batches or due to re-slurrying. The batch of CBZ dihydrate used throughout the drying study via the DVS reported to this point, was generated using the standard procedure detailed in **Section 2.3.1.2**. Using this method, crystals were considered ‘Medium’ in size. Smaller crystals of CBZ dihydrate had been previously generated (**Section 2.3.1.3.1**) for use in the stability study using the Cuspor systems (**CHAPTER 4**). These crystals were originally generated via the standard procedure and then milled using a hand-held mill. As this method proved successful, it was decided to use the remainder of this batch, re-

slurried in water and filtered in such a way to give a 'wet' sample cake for use in the DVS. Prior to carrying out this work, TGA analysis was carried out on this previously generated sample to ensure the dihydrate was still present. **Figure 5.35** showed a weight loss of 11.857%. This was less than the theoretical ~13% expected for the dihydrate. However, slight differences in weight loss are common as the instrument equilibrates prior to the start of the actual analysis. It was concluded that the dihydrate was still present so the re-slurrying for a 'wet' sample could be carried out.

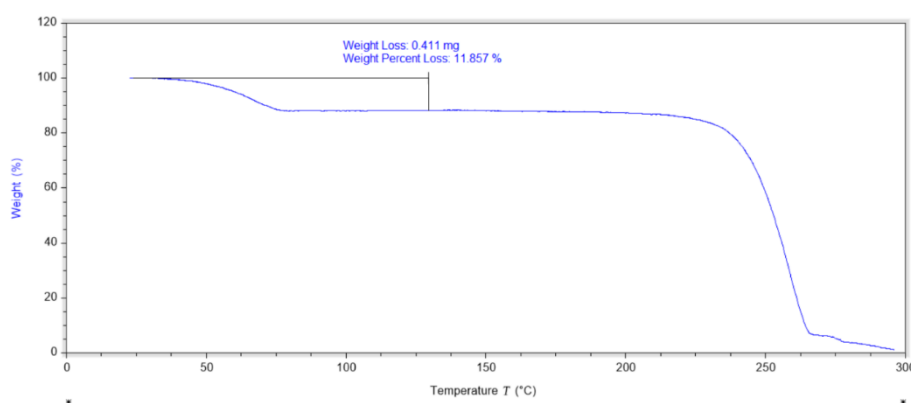


Figure 5.35: TGA analysis on previously prepared batch of CBZ dihydrate with reduced particle size.

For the large crystals, a thermocycling method was used as detailed in **Section 2.3.1.3.3**. PSD data was collected for each sample using a Malvern 3000 (**Section 2.4.6**). Each sample was analysed in duplicate, and three measurements taken for each sample, resulting in six data points per sample. **Table 5.3** shows the d10, d50 and d90 averages across the six data points for each batch of CBZ dihydrate. The Large crystals were confirmed as being larger than the other batches for d10, d50 and d90. The Medium particles had a larger d90 value when compared to the Small particles. Differences between d10 values were determined as statistically significantly different with a p value < 0.05, while the differences between d50 values were not deemed statistically significant with

a p value > 0.05. All PSD values for each sample can be found in **Appendix A**.

The particle size distribution of a sample can significantly impact the drying kinetics. When it comes to drying, smaller particles tend to dry more quickly than larger particles due to their larger surface area-to-volume ratio. This means that a sample with a narrower particle size distribution, consisting mainly of smaller particles, will generally dry more rapidly compared to a sample with a wider size distribution that includes larger particles. Furthermore, particle size can influence the formation of agglomerates or aggregates during drying. Larger particles are more likely to form agglomerates, which can hinder the evaporation process and prolong drying time. Conversely, a sample with a more homogeneous size distribution will have fewer opportunities for agglomeration, facilitating faster drying kinetics. Therefore, controlling and optimizing the particle size distribution is crucial in drying processes to ensure efficient and uniform drying rates.

Table 5.3: PSD data for the Small, Medium and Large CBZ dihydrate samples.

Particle size distribution parameters (average $\pm$ std. deviation)			
Sample	d10 (μm)	d50 (μm)	d90 (μm)
Small	2.86 $\pm$ 0.06	12.42 $\pm$ 1.04	116.25 $\pm$ 10.94
Medium	2.53 $\pm$ 0.05	11.83 $\pm$ 0.76	318.17 $\pm$ 79.23
Large	33.48 $\pm$ 2.34	599.33 $\pm$ 32.26	1145.00 $\pm$ 86.55

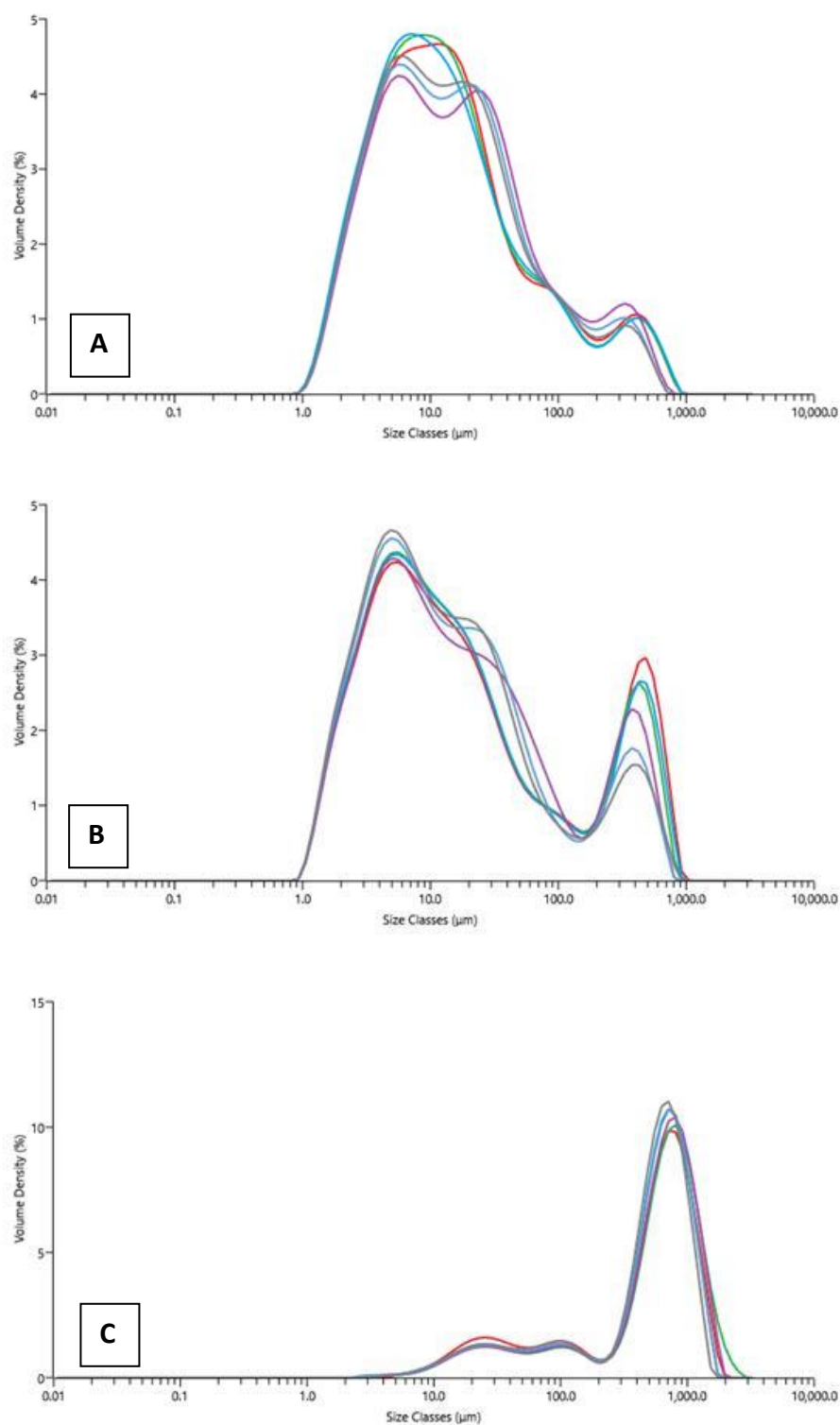


Figure 5.36: PSDs of all **(A)** Small, **(B)** Medium, and **(C)** Large CBZ dihydrate samples.

Figure 5.37 shows the full profiles of each sample analysed in the DVS at 40% RH and 40 °C. Showing the drying profiles (1), period of

hydrate stability (2), where conditions allowed and the dehydration profiles (3), along with the start of the 0% RH step (*).

An initial examination of the graph shows that the Large crystals dried faster than the Small and Medium particles. Small and Medium particles had very similar drying times, with their drying curves overlaying closely.

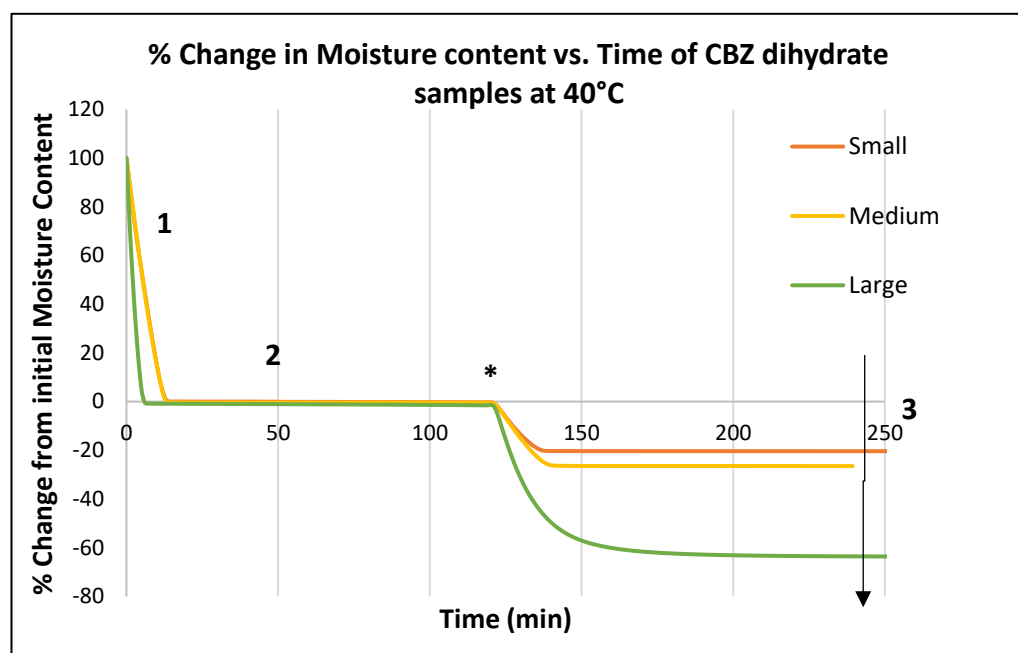


Figure 5.37: % Change in Moisture Content vs. time of CBZ dihydrate samples of increasing particle size at 40% RH and 40°C. * Denotes the start of the 0% RH step.

5.3.1.3.1. Effects of particle size on the drying profile of CBZ Dihydrate

To examine the drying profiles of each system, **Figure 5.38** shows a zoomed in section from the original profiles shown in **Figure 5.37**.

The drying times of both the Small and Medium crystals look to be ~14 min. The drying curves of both samples are nearly identical and, with d50 values shown in **Table 5.3**, not being statistically significant from one another, this could be the reasoning for this.

The Large crystals had a drying time half that of the smaller crystals at only ~7 min. Normally, it would be expected that powder comprised of smaller particles, giving a larger surface area would have faster drying times. However, that is not the case with the CBZ samples. As previously mentioned, the wider size distribution of both the Small and Medium particles could be reason for the longer than expected drying times, suggesting the presence of larger particles, even in small amounts.

The d90 values across the batches of Small, Medium and Large crystals showed the largest differences and therefore, called into question the effect of d90 values on drying specifically. While the D90 value provides information about the particle size distribution, it does not directly determine the drying time. However, it can indirectly influence the drying process in the following ways:

1. Surface Area: Particles with a smaller D90 value tend to have a larger surface area per unit mass compared to particles with a larger D90 value. As a result, smaller particles may dry faster than larger particles due to the increased surface area available for evaporation.
2. Moisture Content: differing initial moisture content levels can also affect drying times. Particles with higher moisture content will take longer to dry compared to particles with lower moisture content. Larger d90 could result in longer time take for moisture to move from within the lattice, to the surface for removal.

It's important to note that the drying time is influenced by numerous factors beyond particle size, such as ambient conditions (temperature, humidity, airflow), particle shape, particle composition, and drying method (e.g., convective, conductive, or radiative drying). Therefore, while D90 can have an indirect impact

on drying time, it should be considered alongside other relevant parameters to accurately assess and predict the drying behaviour of different particle batches.

Another topic that these results raised was the presence of possible defects on the Large particles. The presence of defects can alter the surface properties and morphology of the crystals, affecting their interaction with the surrounding environment during drying. In large crystals, defects can include planar defects (such as grain boundaries, twin boundaries, and stacking faults) and volume defects (such as voids or inclusions). These defects can arise due to growth conditions, thermal stresses, impurities, or mechanical deformation during crystal growth or subsequent processing. ^[123]

The way defects can affect drying include:

- Surface area: Crystal defects, such as dislocations or grain boundaries, can increase the effective surface area of the particles. This increased surface area provides more sites for moisture evaporation, leading to enhanced drying kinetics. The defects effectively act as additional drying sites compared to a perfect crystal lattice.
- Pore formation: Crystal defects can contribute to the formation of pores or voids within the particles. These pores can serve as pathways for moisture to escape during drying, facilitating faster moisture removal and consequently accelerating the drying kinetics.
- Capillary action: Crystal defects can affect the capillary properties of the particles. Capillary action is the phenomenon in which liquid is drawn into small spaces or pores due to intermolecular forces. Defects can modify the surface energy and wetting behaviour of particles, influencing the capillary action during drying. This can

impact the rate at which moisture is transported and evaporated from the particles.

- Agglomeration: Crystal defects can influence the tendency of particles to agglomerate or stick together during drying. The presence of defects can alter the surface energy and adhesive forces between particles, potentially leading to increased agglomeration. Agglomerated particles have reduced surface area exposed to the drying environment, which can impede moisture evaporation and slow down drying kinetics. ^[123]

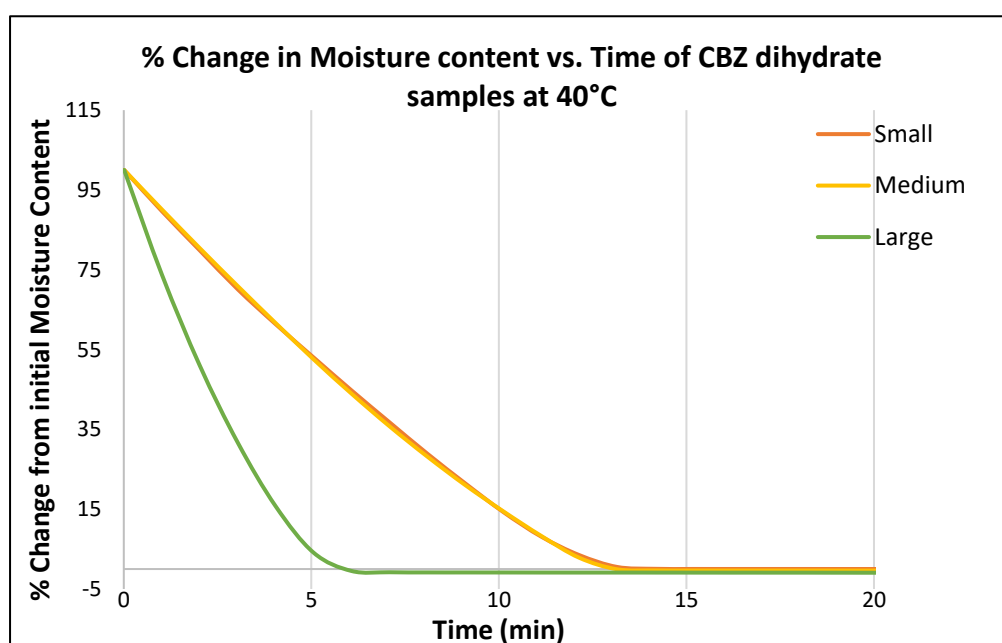


Figure 5.38: % Change in Moisture Content vs. time for CBZ dihydrate samples of increasing particle size at 40% RH and 40 °C, showing the drying profiles.

5.3.1.3.2. Effects of particle size on hydrate stability of CBZ Dihydrate

This section will examine the effects of PSD on hydrate stability.

Figure 5.39 shows a zoomed in portion of the graph shown in **Figure 5.37**.

Small and Medium sized crystals reach 0% M.C. in similar timeframes. However, post drying, differences were seen. After ~60 min, at 40% RH, Small sized crystals begin to dehydrate, although dehydration is minimal, with ~1% M.C. lost prior to the 0% RH step. While minimal, the water of hydration is affected and the stoichiometric amount of water for the dihydrate is no longer present. Medium sized crystals show no additional loss of % M.C. after the initial drying phase and the dry dihydrate, when produced via the standard procedure, is stable for the remainder of the time examined when held at 40% RH, with dehydration only commencing once the 0% RH step begins. The % M.C. for the Large particles falls slightly below the x-axis after the initial drying of loosely bound moisture region. It is likely that data for this experiment began being saved a small time after drying had begun and the true starting weight was not obtained, resulting in this slight discrepancy for the calculated % M.C. over time. However, based on the stability (lack of reduction in % M.C.) shown in the profile, it is likely a region of stable, dry dihydrate. Stability of the Large crystals begins to change, slowly, at ~40 min, with reduction in moisture content being related to dehydration. Approximately 2% M.C. is lost before the 0% RH step begins. Dehydration at 40% RH is very slow but does occur, meaning the dihydrate is being adversely affected.

Coupling the DVS data with the PSD data in **Table 5.3** again, as d50 values of Small and Medium particles were not considered statistically different, the difference in hydrate stability between Small and Medium particles is difficult to explain. While all samples show periods of hydrate stability, Medium dihydrate particles generated by the standard method (**Section 2.3.1.2**) was the only sample which maintained hydrate stability after the initial drying

phase, and dehydration only occurred once the 0% RH step was implemented.

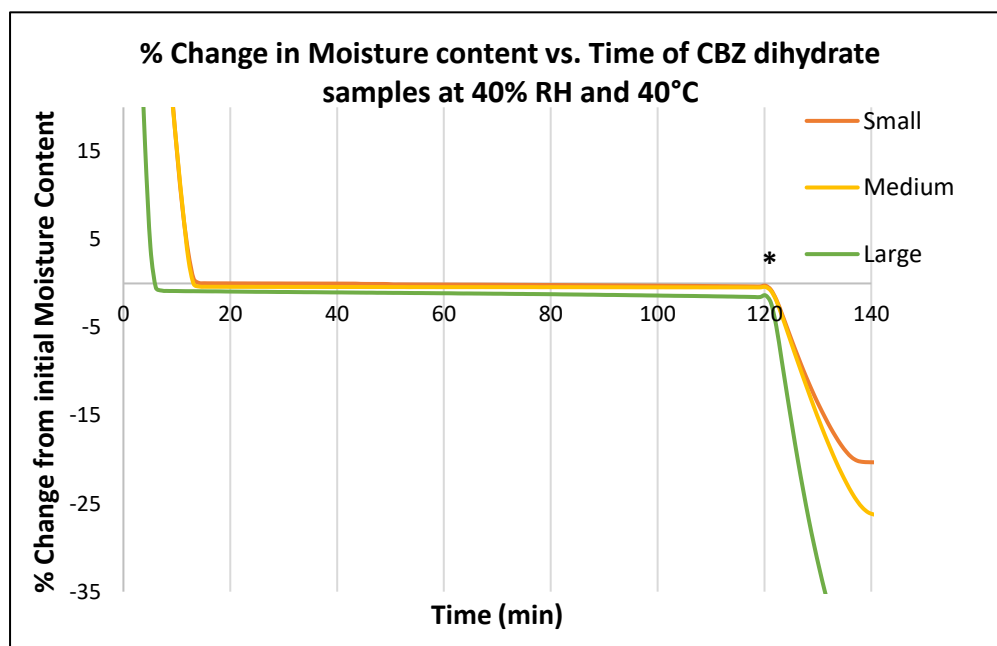


Figure 5.39: % Change in Moisture Content vs. time for CBZ dihydrate samples of increasing particle size at 40% RH and 40 °C, showing periods of hydrate stability (when % moisture content equals 0%). * denotes where the 0% RH step.

5.3.1.3.3. Effects of particle size on the dehydration profile of CBZ Dihydrate

As mentioned at the top of **Section 5.3.1.3**, adjustments made to the starting % M.C. to aid comparisons altered true end points but the stability seen from ~220 min demonstrates the complete removal of all moisture in each sample. **Figure 5.40** shows a zoomed in section of **Figure 5.37**, more specifically, the dehydration profiles of each sample.

From the start of the 0% RH step, dehydration takes ~20 min for the Small crystals sample and ~25 min for the Medium sized crystals sample. Finally, for the Large crystals, the % M.C. consistently decreased up to ~220 min, meaning the full dehydration of this sample took ~100 min. This is a much longer dehydration time than

the other samples. Also, when compared with samples subjected to the highest % RH level at 40 °C (**Section 5.3.1.1.3**) and 30 °C (**Section 5.3.1.2.3**), the dehydration time was longer. It appears that, while the excess, loosely bound moisture was removed quickly, the tightly bound water of hydration took longer to navigate the exit routes of the larger crystals.

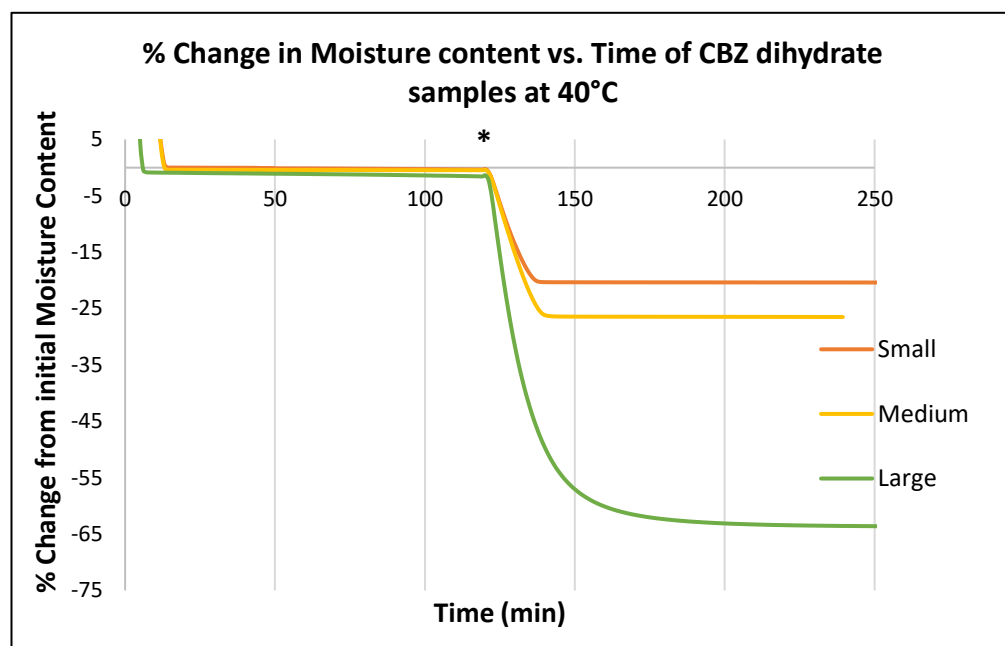


Figure 5.40: % Change in Moisture Content vs. time for CBZ dihydrate samples of increasing particle size at 40% RH and 40 °C, showing the dehydration profiles. * denotes where the 0% RH step.

5.3.1.4. Investigating hydrate stability

Previous sections which discussed periods of hydrate stability showed conditions and time points in the DVS studies where the dry dihydrate could be found. However, extended stability of the dihydrate was not investigated further. In this section, samples underwent longer experiments to see how the dihydrate behaves under longer hold times of 12 hrs. Experiments were carried out at 40 °C and at 40% RH and 60% RH. 40% RH was chosen as the starting point for this as it was the first set of conditions which showed

significant stability of the hydrated form in the DVS studies detailed above.

5.3.1.4.1. 12 hour holds at 40% and 60% RH

Experiments were set up in the same way as previously, except the initial step was held for 12 hrs and then followed by the 0% RH step.

Figure 5.41 shows the results, where the differences due to the levels of % RH are clear. At 40% RH, we see the initial slope for the drying of loosely bound moisture, then stability of the dry dihydrate. This stability begins to fall away at approximately 150 min and dehydration slowly begins. The rate of dehydration is clearly impacted by the 40% RH environment, however dehydration continues slowly until 720 min. Here the 0% RH step begins, and its effect is obvious with dehydration then taking ~15 min to remove the remaining moisture. At 60% RH, post drying, the dry dihydrate remains stable for the remainder of the 720 min step. Changes are only seen when dehydration is once again induced by the 0% RH step. This is another instance where the consistent dehydration time is shown at 20-30 min for samples at 40 °C, as discussed in **Section 5.3.1.2.3**. Drying at 40 °C and 60% RH looks to be an advantageous drying protocol for CBZ dihydrate, as it dries the sample quickly and provides the dry dihydrate which remains stable for a long time.

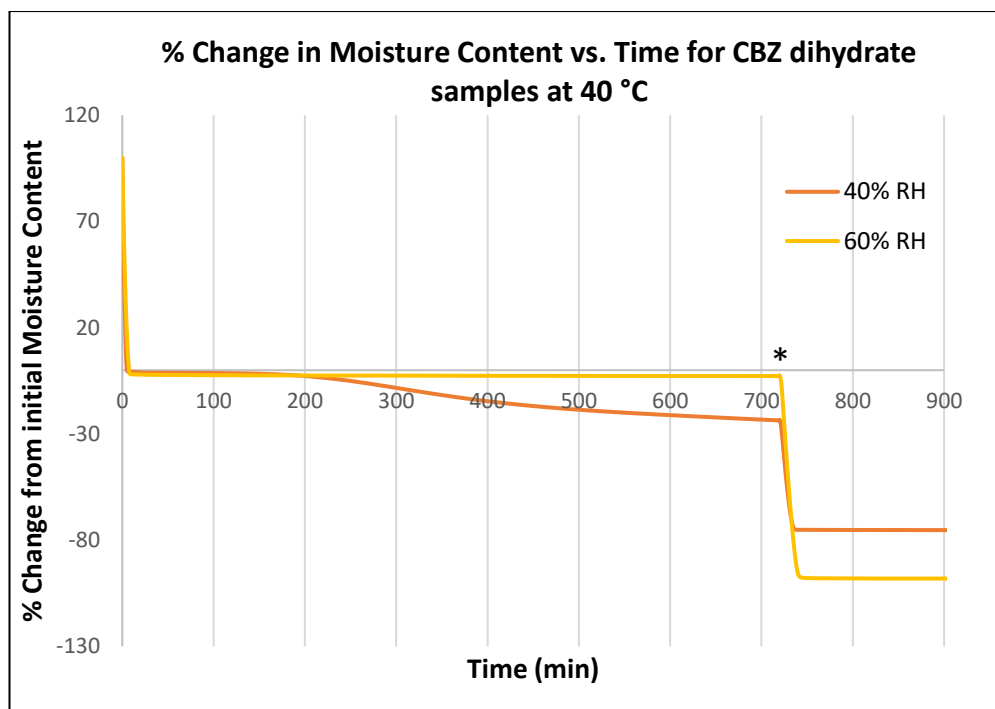


Figure 5.41: % Change in Moisture Content vs. time for CBZ dihydrate samples at 40 °C, with long holds (12 hrs) at 40% RH and 60% RH. * denotes the start of the 0% RH step.

5.3.1.4.1.1. Solid-state analysis of post DVS samples

As mentioned in **Section 5.3.1.1.4**, small sample size meant choosing the most beneficial types of analysis and, to examine the effects of long hold times, samples were analysed by XRPD.

5.3.1.4.1.1.1. XRPD Analysis

Neither sample, post DVS, had peaks in common with the pre-drying study, 'wet' dihydrate (see **Figure 5.42** for the overlay of the traces). While most peaks corresponded to peaks in the anhydrous starting material, there was a lost peak at $\sim 10^\circ 2\theta$ and two additional peaks which could not be accounted for, as was seen in **Section 5.3.1.1.5.1**. The missing peak could be more likely due to the loss in resolution for these samples. The additional peaks are found at $\sim 16.5^\circ 2\theta$ and $\sim 18^\circ 2\theta$. It is still unconfirmed as to what has caused these peaks. The reduced resolution of these samples is

likely due to the smaller sample size. Sample preparation is optimized as much as possible, with efforts to concentrate the sample into the middle of the holder. Resolution shown here, is the best that could be achieved with these samples.

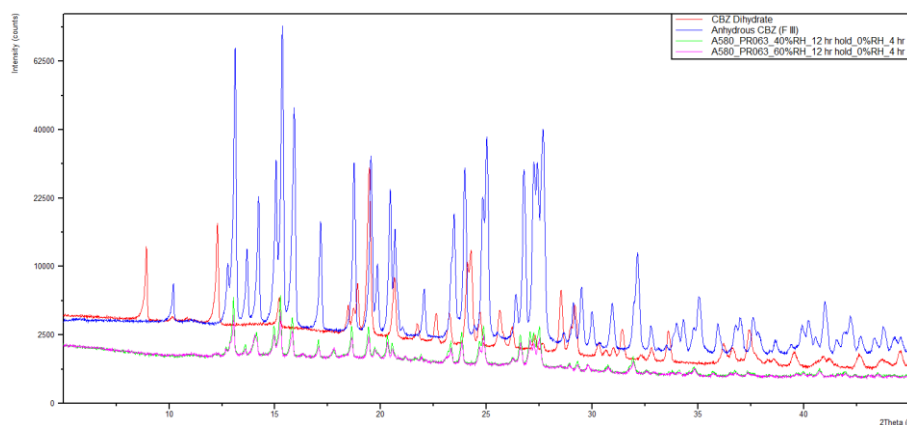


Figure 5.42: XRPD overlay of CBZ dihydrate (red), anhydrous Form III starting material (blue), post DVS at 40% RH for 12 hr, 0% RH for 4 hr (green) and post DVS at 60% RH for 12 hr, 0% RH for 4 hr (pink).

5.3.1.5. Investigating the effects of increased sample size

In this section, the effect of increased cake depth on drying time was investigated. Experiments were carried out at 40 °C, 40% RH for 2hr, followed by 40 °C, 0% RH for 2hr. Samples were prepared in the same way as detailed in **Section 2.8.1** but with an increased sample weight. The same flat surface area of the pan was covered here, but with an increased weight and depth of sample.

Increasing the sample amount resulted in increased drying time (as shown by the overlay of results with the previously analysed sample at the same conditions – see **Figure 5.43**). Sample sizes within the DVS are considered very small when compared to sample cakes dried in lab ovens. Small sample cakes, such as those examined from the DVS, might not be ideal for studying the differences in mass transfer during drying. Despite this, when looking at DVS data on its own, it is beneficial information that cake

depth affects drying times, which stresses the importance of standardising sample preparation for collecting this type of drying data.

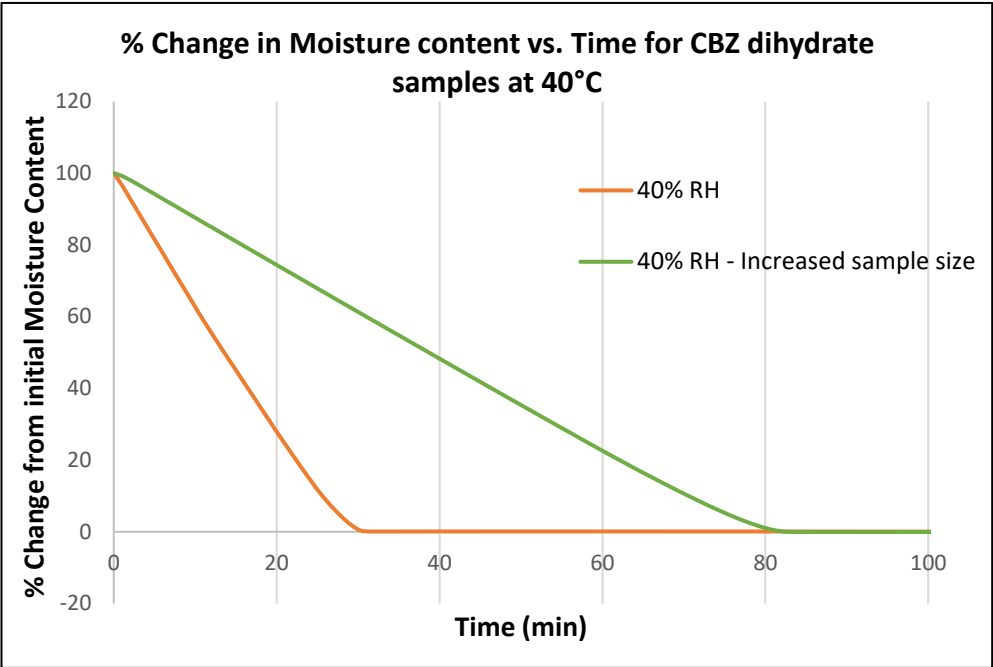


Figure 5.43: Drying curves of CBZ dihydrate samples at 40 °C and 40% RH, comparing the drying times with increased sample weight.

5.3.2. THEO Monohydrate

After crystallising the sample, a sample with <50% total moisture content was considered to be an appropriately wet starting sample for DVS drying studies. Moisture Content analysis was undertaken in duplicate, and results can be seen in

Table 5.4 below:

Table 5.4: % Moisture Content values of THEO monohydrate.

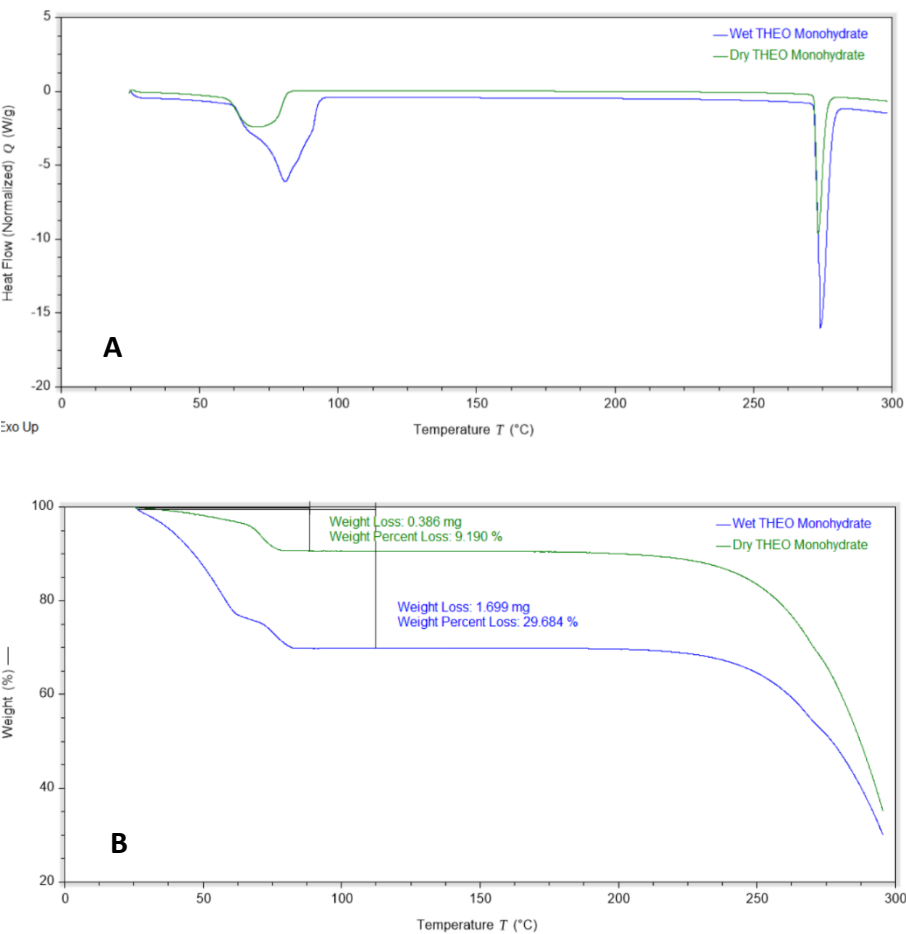
PR058 – Wet THEO Monohydrate	
S01_1 - %	42.11
S01_2 - %	45.26
<i>Average</i>	<i>43.685</i>
STDEV	1.575
% RSD	3.605

A small amount of this sample was left to dry under ambient conditions to confirm the presence of the monohydrate. **Figure 5.44** shows a summary of the solid-state analysis completed. The DSC thermogram (**Figure 5.44 (A)**) shows the peak of hydration prior to 100 °C and the characteristic Form II melt at ~270 °C. This trace compares well with previously generated batches of THEO Monohydrate. Similarly, TGA results (**Figure 5.44 (B)**) show a weight loss of $9.19 \pm 0.08\%$ at temperatures below 100 °C which correlates well to the theoretical loss of the monohydrate, being 9.09%.

The ‘wet’ sample shows a total weight loss of 29.684% from a single TGA measurement. There are two steps in the TGA profile with the first step roughly equating to the excess, loosely bound moisture and the smaller, second step being that of the water of hydration. Finally, XRPD analysis (**Figure 5.44 (C)**) shows an overlay of the wet THEO monohydrate and a sample of the ambiently dried THEO monohydrate. XRPD results showed both patterns overlay with no additional or lost peaks for either sample. The solid-state analysis

confirms the presence of the monohydrate form and Moisture Analysis (

Table 5.4), shows the additional, loosely bound moisture present in the sample to be used in the drying studies via DVS.



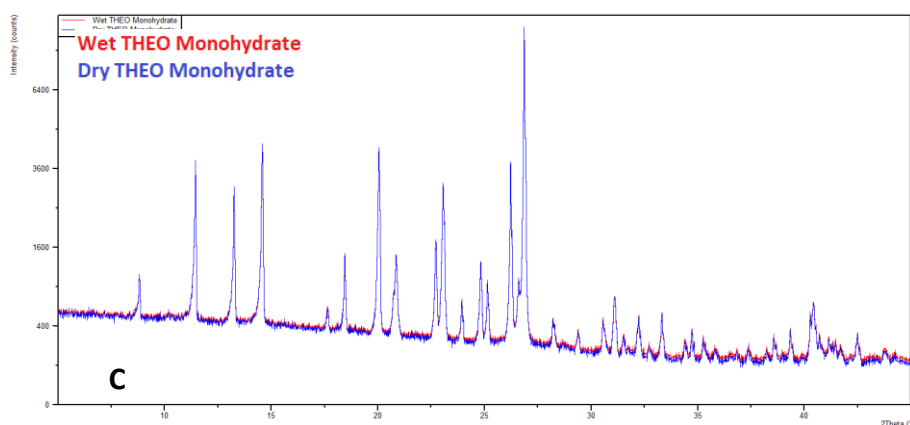


Figure 5.44: A) DSC thermogram, B) TGA, C) XRPD of the dried THEO monohydrate.

5.3.2.1. Effects of relative humidity on wet THEO monohydrate at 40 °C

Figure 5.45 shows the % M.C. versus Time curves for wet THEO monohydrate at 40 °C. Each sample's data set was adjusted to give the same starting point, to allow for comparisons. Once again, the graph can be broken down into three sections: (1) drying of excess, loosely bound moisture, (2) region of hydrate stability after drying excess moisture and (3) dehydration (removal of water of hydration) due to unfavourable conditions or the introduction of a 0% RH step.

In samples with no hydrate stability, i.e., those analysed at 0, 10 and 20% RH, a point of inflection is evident and clearly demonstrates a change in drying rate in the periods between drying the loosely bound to tightly bound moisture (water of hydration). For experiments conducted at $\geq 40\%$ RH, periods of hydrate stability are evident, prior to the start of the 0% RH step. For THEO monohydrate, the 0% RH step was set for 120 min for 40 °C samples and 600 min for samples analysed at 30 °C. Initial DVS experiments

using dry THEO monohydrate found that the water of hydration was consistently fully removed after these times.

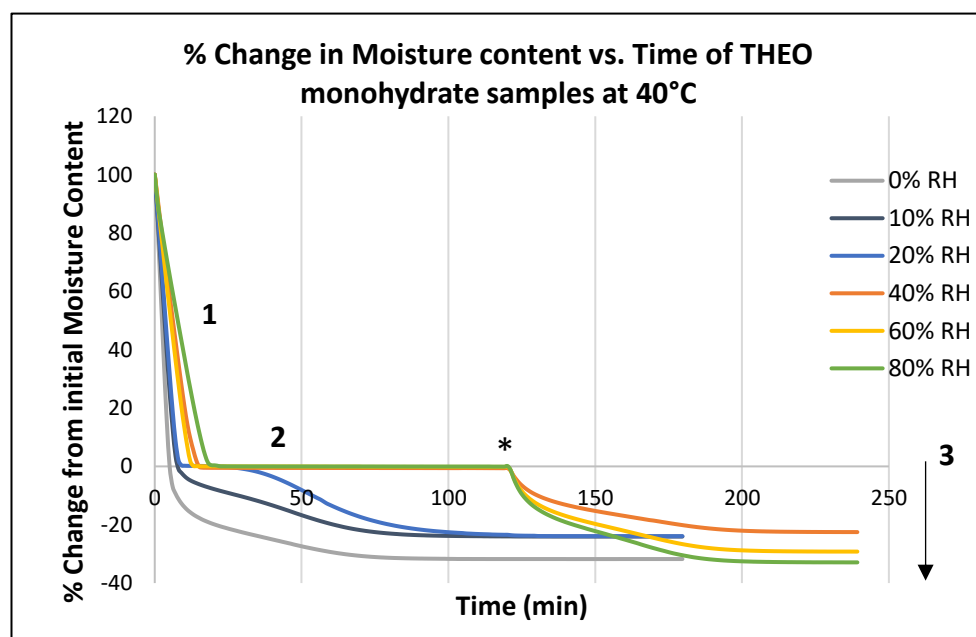


Figure 5.45: % Change in Moisture Content vs. time for THEO monohydrate at 40 °C and varying %RH. * denotes the start of the 0% RH step.

5.3.2.1.1. Effects of relative humidity on the drying profile of THEO monohydrate at 40°C

To examine and compare the drying of each sample, **Figure 5.46** provides a zoomed in portion of **Figure 5.45**, showing the drying curves of the loosely bound moisture under increasing levels of % RH.

The drying times increased with the level of relative humidity, except for the sample at 40% RH. Removal of the moisture in this sample took longer than for the 60% RH system, which was unexpected based on the trends of the other samples. Drying times

for this sample at 40 °C, across all RH conditions, were short, ranging from 5 min at 0% RH to 19 min at 80% RH. Triplicate analyses at 40 and 60% RH were more closely examined (**Figure 5.47**). The first sample analysed showed the only instance of the 60% RH sample drying faster than the 40% RH sample. The second and third samples showed different results, with the 60% RH sample taking longer to dry. The 60% RH sample, across all three samples showed the greatest differences in drying times. While efforts were consistently made to take representative samples for each DVS run, samples subjected to 60% RH increased in drying times over the triplicate runs and it is possible that errors were made when sampling. The first sample was taken without mixing and taken from close to the top of the bulk container and subsequent samples were taken post mixing and from deeper within the sample container. Improper sample preparation was probably the reason for the out of trend finding for the original (first) sample.

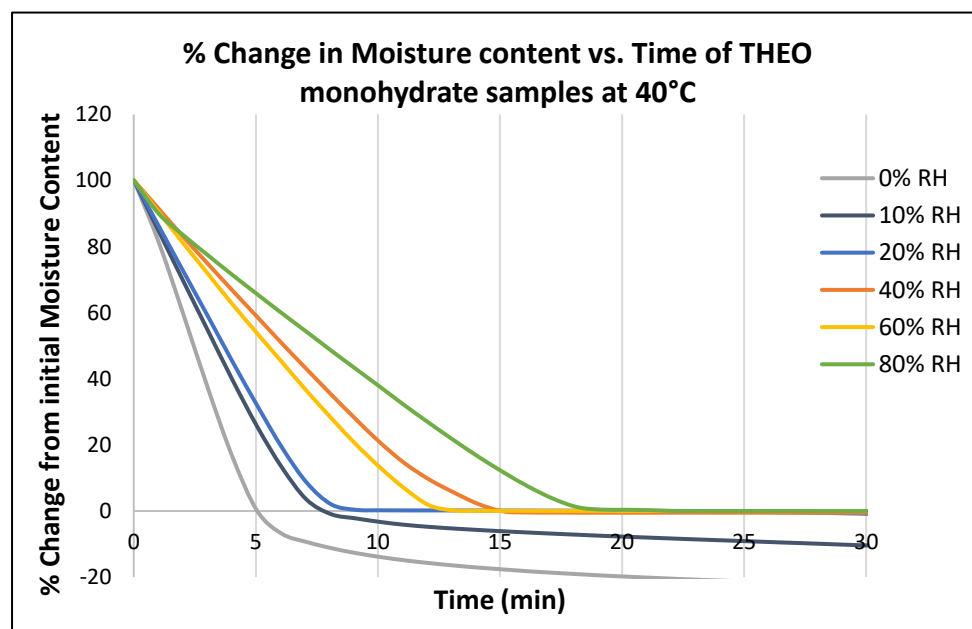


Figure 5.46: % Change in Moisture Content vs. time for THEO monohydrate at 40 °C and varying %RH, showing drying profiles.

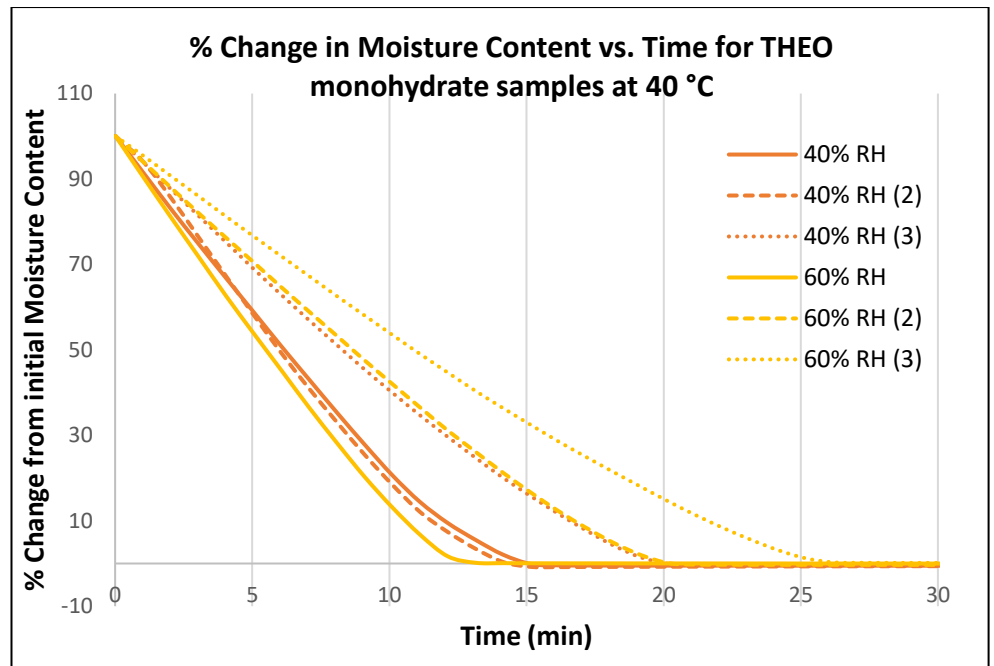


Figure 5.47: % Change in Moisture Content vs. time for triplicate THEO monohydrate samples analysed at 40% and 60% RH, showing drying profiles.

Taking a closer look at **Figure 5.46**, results show that dehydration smoothly follows the drying of the excess moisture region for the 0 and 10% RH samples. An inflection point can be seen. At relative humidities higher than 10% RH, each curve remains on the x-axis (0% M.C.) for a differing amount of time, suggesting the dry monohydrate is present.

Another way to examine this data, is to generate a traditional drying rate curve, which is shown below in **Figure 5.48**. Data shows that the drying rate decreases with increasing levels of relative humidity, which is an expected outcome. At RH levels $\geq 20\%$, graphs pass through the origin, with 0% M.C., showing evidence of the dry monohydrate prior to further removal of the tightly bound moisture. For these samples, the drying rate sharply increases correlating to the induced dehydration caused by the applied 0% RH step, denoted with an asterisk in **Figure 5.45**. When compared to the samples subjected to $<20\%$ RH, where a constant, gradual

decrease in drying rate is seen, drying rate curves do not pass through the origin, with the drying rate decreasing with % M.C. of the sample until no moisture remains. This further backs up the conclusion that at lower RH levels, hydrate dehydration immediately follows on from drying of loosely bound moisture.

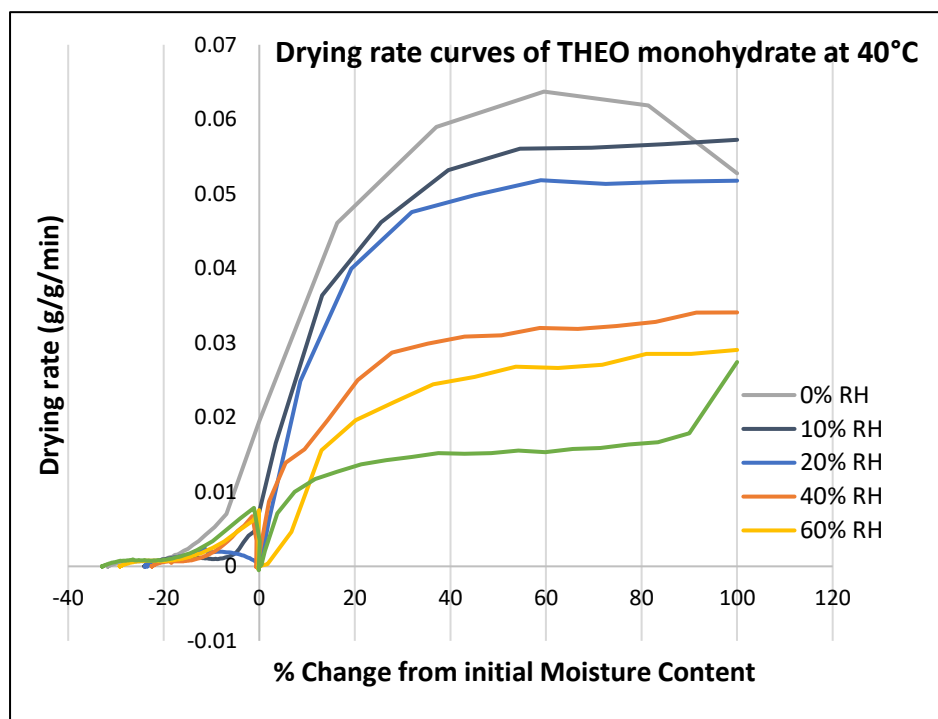


Figure 5.48: Drying rate curves for THEO monohydrate at 40 °C, with increasing % RH environments.

THEO monohydrate dried quickly across all RH levels. Examining the stability of the dry hydrate will provide more information on the robustness of the drying protocol and whether the dry hydrate can be retained.

5.3.2.1.2. Effects of relative humidity on hydrate stability after drying of THEO monohydrate at 40 °C

To examine hydrate stability, where the sample is at 0% M.C., **Figure 5.49** shows a zoomed in portion of the graph shown in **Figure 5.45**.

Increasing the % RH environment gave an increase in hydrate stability, with stability in some samples only being jeopardised when induced by the introduction of the 0% RH step. At 0 and 10% RH, results show there is no hydrate stability region. THEO monohydrate cannot be successfully dried at these conditions so as to retain the hydrate form. Increasing to 20% RH sees the dried monohydrate being present after ~9 min and remaining stable for a further 10 min before dehydration begins. All the water of hydration is lost prior to the 0% RH step starting at 120 min. While a small period of stability can be seen at 20% RH, this is not robust for a good drying protocol. However, for samples at $\geq 40\%$ RH, once the excess moisture is removed the monohydrate remains stable until the 0% RH step begins.

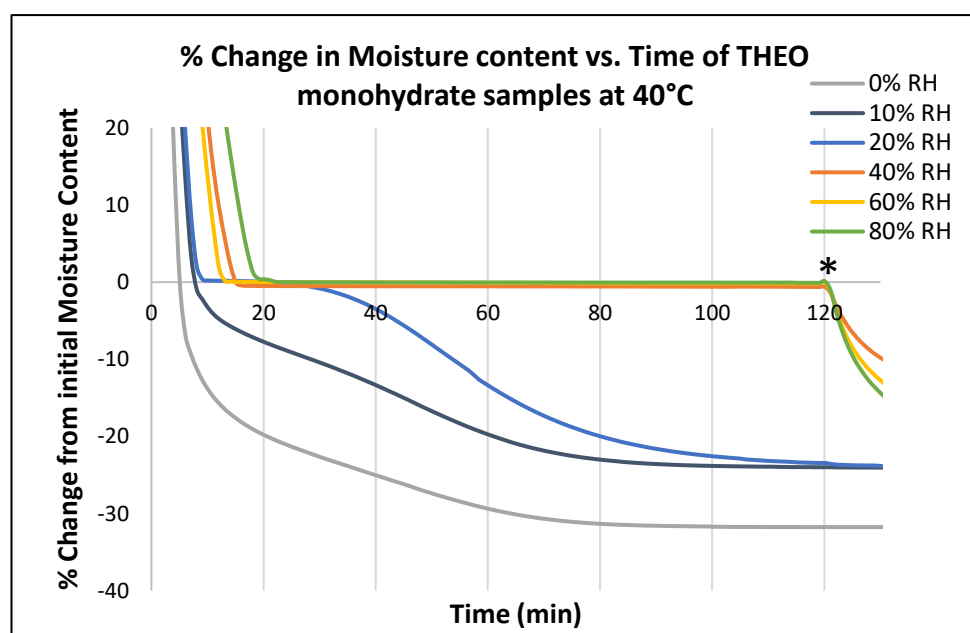


Figure 5.49: % Change in Moisture content vs. time for THEO monohydrate at 40 °C and varying %RH, showing periods of hydrate stability where conditions allow. *denotes the start of the 0% RH step.

To confirm the presence of the dry monohydrate when the % M.C. is zero, an experiment was set up using the DVS at 60% RH, 40 °C and holding at these conditions for 60 min, after which time a

sample which had 0% M.C. (based on **Figure 5.49**) was expected to be present. The sample was then analysed by TGA, **Figure 5.50**, which showed a weight loss of 8.99% occurred, which equates to the monohydrate being present.

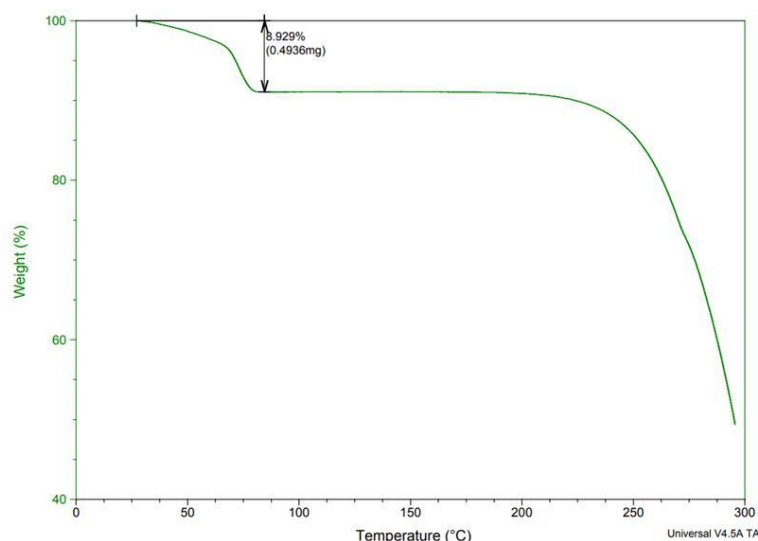


Figure 5.50: TGA Analysis of a THEO monohydrate after 60 min at 60% RH, 40 °C.

Knowing the boundaries for the stability of the hydrated form provides useful data for the recovery of the correct product (i.e., dried but not dehydrated hydrate) and the time points at which to achieve this.

5.3.2.1.3. Effects of relative humidity on the dehydration profile of THEO monohydrate at 40 °C

Figure 5.51 shows a zoomed in section of the graph previously discussed, **Figure 5.45**, showing the dehydration profiles.

Below 20% RH, results showed inflection points as the sample moved from wet, to a dried monohydrate to dehydration, with the endpoint and stability in the sample Moisture Content being found after ~90 min. At 20% RH dehydration begins at 20 min and takes a further 100 min for completion. All water in this sample is lost prior

to the 0% RH step beginning. At 40% RH and above, dehydration is only induced once the 0% RH step begins. Dehydration for each of these samples takes ~100 min for stability in the % M.C. to then be seen. Due to adjusting the data of each sample to have the same starting % M.C., end points for each of these samples have differences.

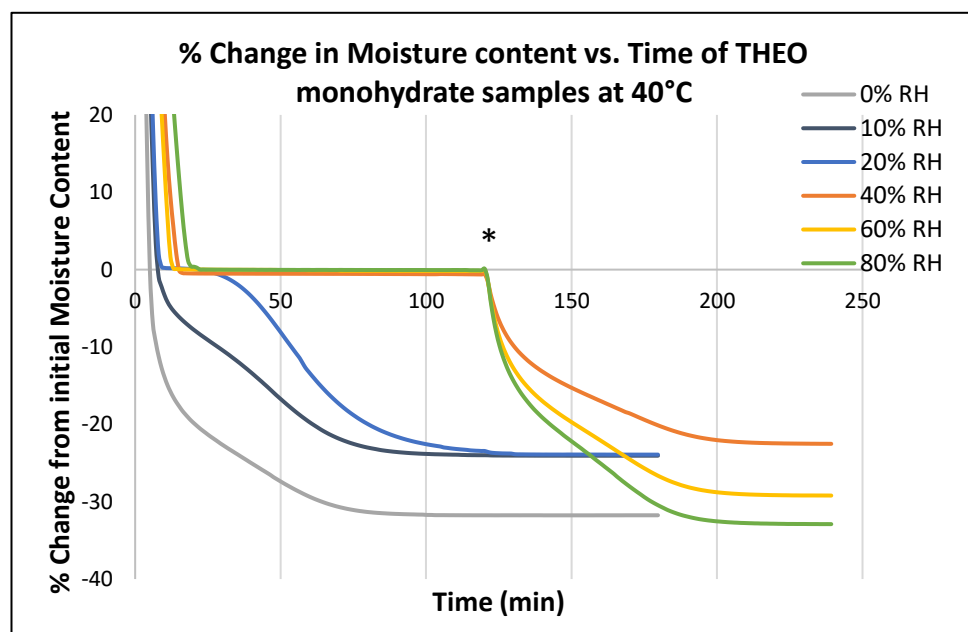


Figure 5.51: % Change in Moisture content vs. time for THEO monohydrate at 40 °C and varying %RH, showing the dehydration profiles. * denotes the start of the 0% RH step.

5.3.2.1.4. Solid-state analysis of post DVS, end point samples at 40 °C

The effects of each drying experiment on the THEO monohydrate crystals were investigated through solid-state analysis. DSC, SEM and XRPD analysis was carried out on the samples to determine the forms and any morphology changes post DVS experiments.

5.3.2.1.4.1. DSC Analysis

Post DVS samples were analysed by DSC to see how each set of conditions affected the THEO monohydrate. End point samples, across all conditions, lost the peak of hydration in the DSC and showed only one endotherm. **Figure 5.52** shows the overlay of the end point samples. Each thermogram overlays and shows only the Form II melt, indicative of the anhydrous Form II starting material, indicating this form is present after both loosely and tightly bound moisture are removed.

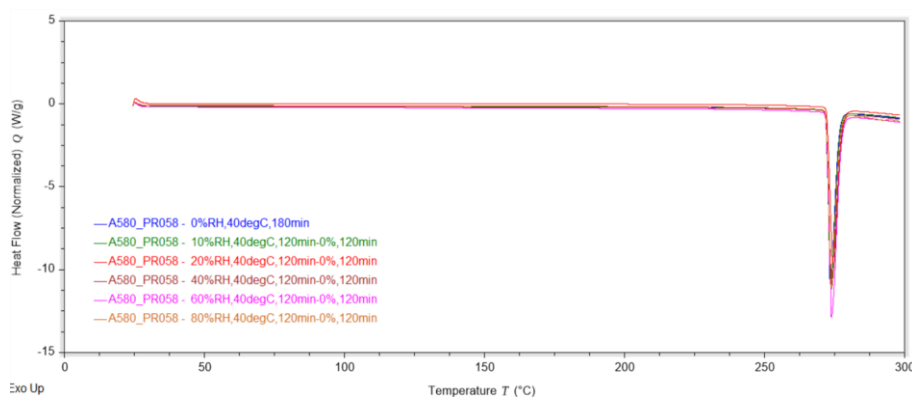


Figure 5.52: DSC overlay of post DVS samples of THEO monohydrate which were analysed at 0, 10, 20, 40, 60, 80% RH and 40 °C.

5.3.2.1.4.2. SEM Analysis – Gold Sputter Study

Like CBZ dihydrate samples, a small study was carried out to determine whether gold sputtering was to be used to aid the comparison between the 'before' images of the wet hydrated crystals and the dried 'after' images of the crystals. This helped determine whether additional sample preparation caused any additional changes to the sample itself or if the quality of imaging was impacted.

Figure 5.53 shows images of wet THEO monohydrate which have been sputtered and **Figure 5.54** shows images of the same wet THEO monohydrate which has not been sputtered. The surfaces of

crystals in both sets of images have similar morphologies and do not look to be affected by the presence or absence of the gold sputtering and then analysis by the SEM. The quality of imaging was not greatly affected either.



Figure 5.53: SEM images of wet THEO monohydrate with gold sputtering.

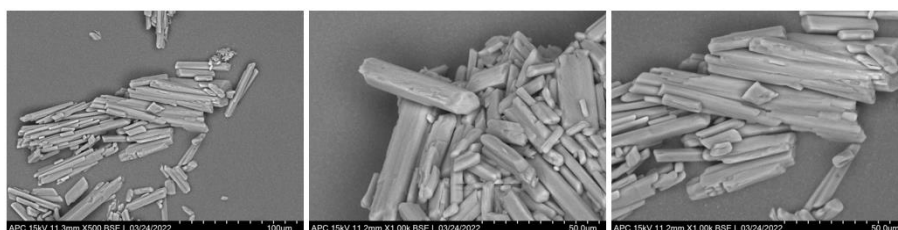


Figure 5.54: SEM images of wet THEO monohydrate without gold sputtering.

Results showed that there were no adverse effects on the samples caused when using the sputterer. In keeping with CBZ dihydrate samples, it was decided to also sputter both the ‘before’ and ‘after’ DVS drying study samples of THEO.

5.3.2.1.4.3. SEM Analysis

Figure 5.55 shows SEM images of the wet THEO monohydrate. Images show rodlike crystals with smooth surfaces which have agglomerated together in larger clusters. Morphological differences between anhydrous and hydrated THEO are more difficult to see, in comparison to CBZ dihydrate, as both materials are rodlike. DSC (**Section 5.3.2.1.4.1**) and later XRPD (**Section**

5.3.2.1.4.4) prove that the monohydrate is the material present in the images below, **Figure 5.55**.

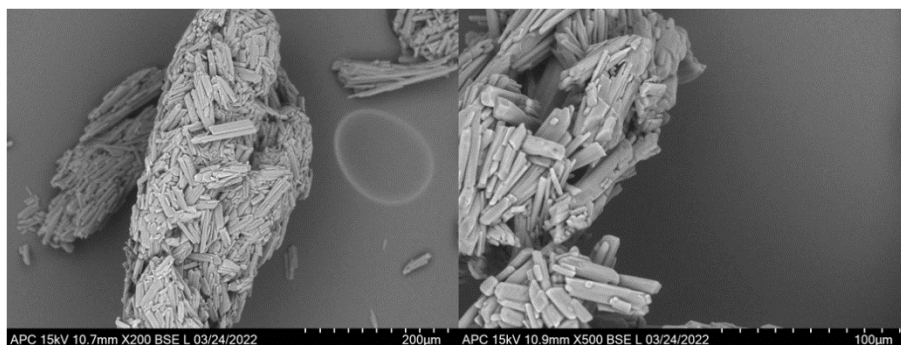
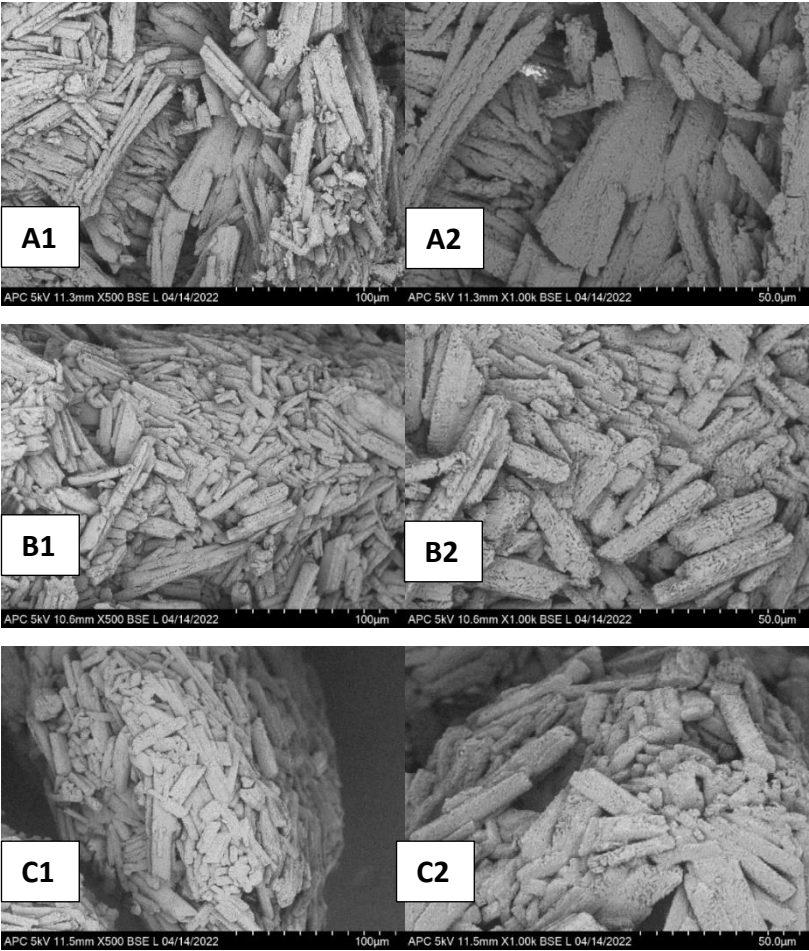


Figure 5.55: SEM images of wet THEO monohydrate prior to drying studies via DVS.

Post DVS analysis samples from 40 °C experiments are shown in **Figure 5.56**. Morphological changes are clear. While the rodlike structures are still present, their surfaces are now rough and rugged. Examining the higher magnification images at x1k, ridges show a tendency to track linearly which is indicative of THEO monohydrate being a channel hydrate. During the DVS experiments, the exit routes for water during the DVS experiments were through these channels. Further proof of this is seen in **Figure 5.57**, where the crystal was positioned slightly perpendicular to the stub, allowing for the hollow spaces to be imaged.

Despite sample preparation being the same, THEO samples proved harder to image at higher magnifications compared to CBZ. There may be several reasons for this, such as poor coating, particle stacking, degradation under the electron beam or an issue with the instrument's filament. As analysis of both CBZ and THEO samples were undertaken over the same period and CBZ samples didn't experience any issues, it's unlikely that the sputterer was the cause. Degradation is also unlikely to be the issue as DSC analysis showed

no thermal events present, other than the characteristic melt of the Form II polymorph across all samples. If evidence of changes were seen due to the environment of the DSC, this could be circumvented within the SEM by using the lower 5 kV beam. The filament was also confirmed to be fit for use. This leaves particle stacking being the possible problem. Particle stacking causes the particles to sway slightly during analysis. Wet THEO monohydrate did not have this issue, only the post DVS samples. A repeated sample of 0% RH, 40 °C post DVS was analysed but had the same issues. Despite these problems in imaging, the changes in morphology due to the drying via DVS are clear.



D1

D2

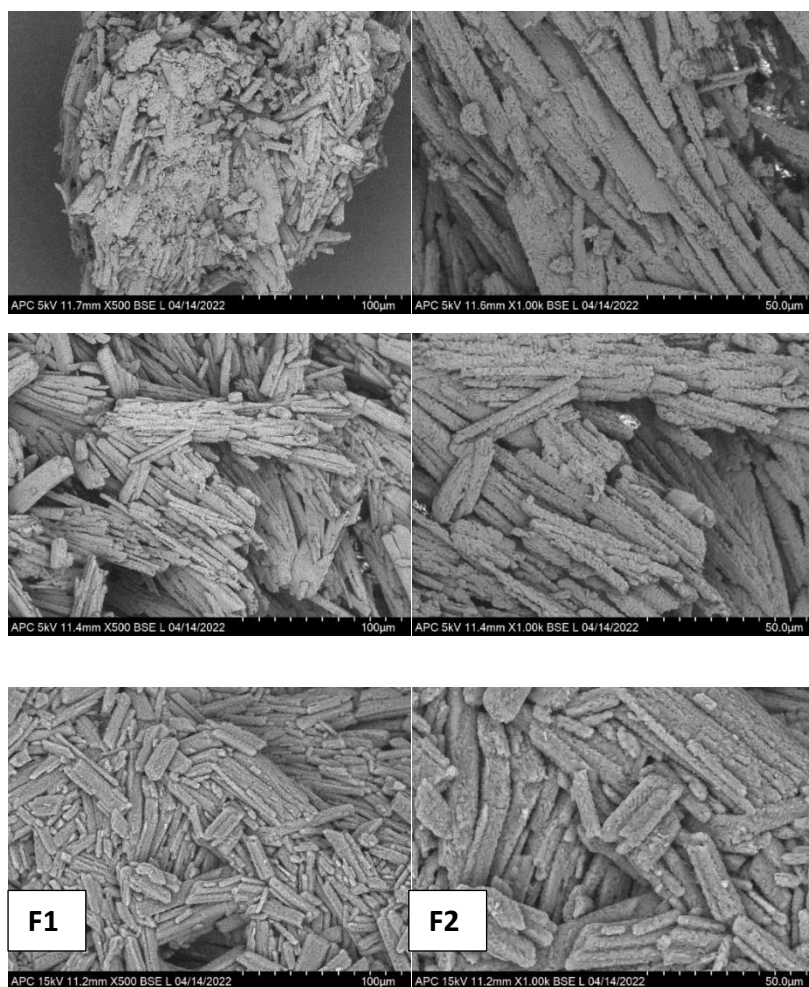


Figure 5.56: SEM images of Post 40 °C DVS drying studies of THEO monohydrate at **A1)** 0% RH, x500, **A2)** 0% RH, x1000, **B1)** 10% RH, x500, **B2)** 10% RH, x1000, **C1)** 20% RH, x500, **C2)** 20% RH, x1000, **D1)** 40% RH, x500, **D2)** 40% RH, x1000, **E1)** 60% RH, x500, **E2)** 60% RH, x1000, **F1)** 80% RH, x500, **F2)** 80% RH, x1000.

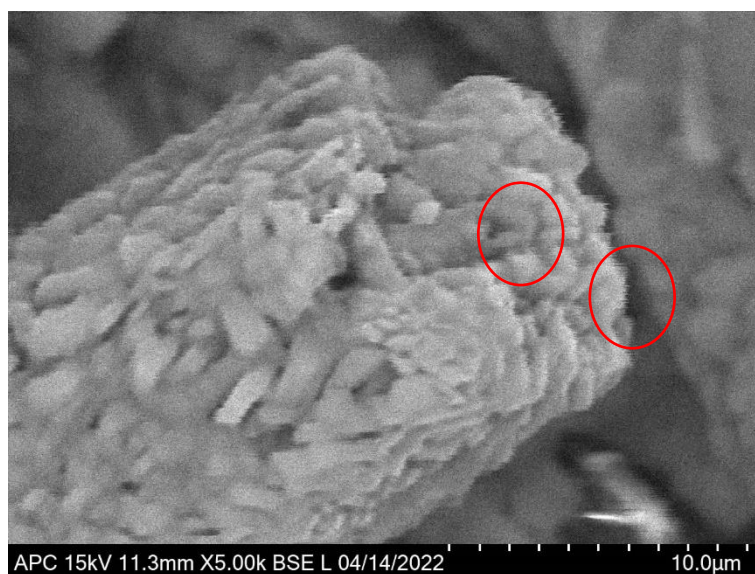


Figure 5.57: SEM image of post DVS THEO showing evidence of channels within the structure.

5.3.2.1.4.4. XRPD Analysis

Figure 5.58 shows the overlay of the anhydrous THEO Form II starting material and a portion of the batch of pre DVS-dried ‘wet’ monohydrate. Clear differences are seen between the samples, and this aids comparison of the end point samples, post DVS.

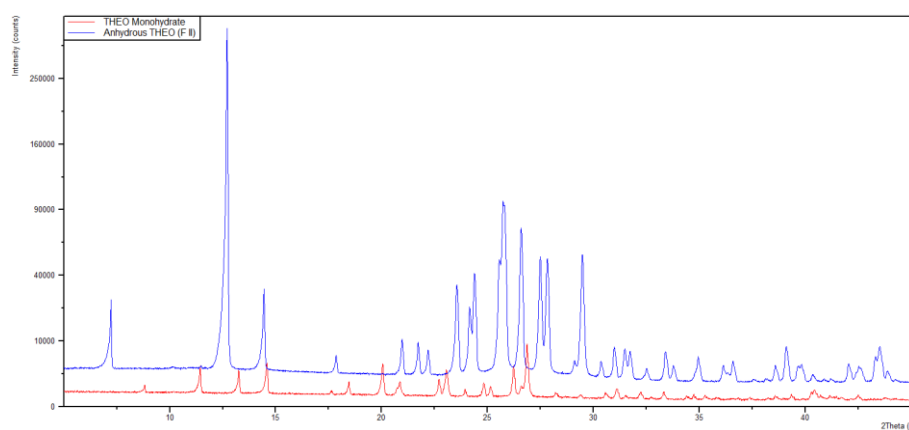


Figure 5.58: XRPD overlay of the ‘wet’ THEO monohydrate (red) prior to the DVS drying studies and the anhydrous THEO Form II starting material (blue).

Figure 5.59, shows the overlay of all post DVS samples at 40 °C, along with the ‘wet’ monohydrate and the starting material. DSC (**Section 5.3.2.1.4.1**) showed a transformation from the monohydrate back to the original starting material and XRPD patterns also confirmed this. No peaks in the end point samples correlated to the monohydrate pattern. The samples from the 10 and 80% RH experiments had very low resolution, likely due to sample preparation due to the amount of sample available. Efforts were made to ensure the sample was concentrated in the middle of the holder, however these samples proved difficult to prepare. For the other samples, all peaks corresponded to peaks in the anhydrous Form II XRPD pattern.

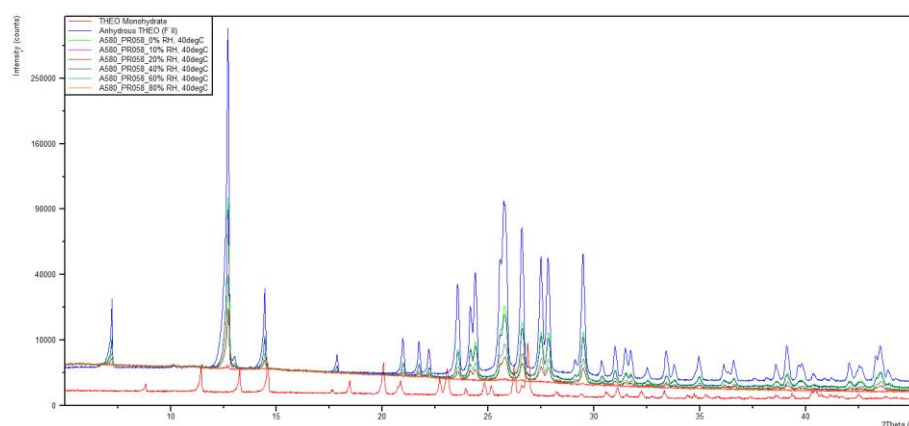


Figure 5.59: XRD overlay of ‘wet’ THEO monohydrate (red), anhydrous THEO Form II starting material (blue), and post DVS samples after analysis at 0% (green), 10% (pink), 20% (brown) and 40% (dark blue), 60% (dark green) and 80% RH (orange) at 40 °C.

At these conditions, it can be said that the dihydrate dries and then dehydrates to give a sample of the anhydrous Form II starting material which confirms what was found through DSC analysis.

5.3.2.1.4.4.1. Non-ambient XRPD Analysis

A non-ambient XRPD chamber (**Section 2.4.5.1**) was used to mimic DVS experiments on the same sample of THEO monohydrate to

track the changes over time. As mentioned in **Section 5.3.1.1.5.1.1**, 0% RH was not achievable in this instrument; 4% RH was lowest value that could be obtained so this was used instead to mimic conditions close to that of 0% RH in other experiments.

5.3.2.1.4.1.1. 4% RH, 40 °C for 4 hr

Shown in **Figure 5.60** are the consecutive XRPD scans of the drying and dehydration of 'wet' THEO monohydrate at 4% RH, 40 °C over 4 hr. Moving through the scans the gradual disappearance of the characteristic peaks of the monohydrate was followed by the appearance of a different crystalline anhydrate phase. **Figure 5.61** showed that the effects of the conditions had already begun to take effect, with differences to the monohydrate form already present. Additional peaks were seen at 9.4, 12.5, 13.7, 15.3, 16.3 and 22.2° 2 θ . From scan 4 the growth of the new peaks is clear, and they continue to grow in intensity up to the last scan. From the literature ^[90], the end point samples trace (**Figure 5.62**) compared well to the metastable anhydrous THEO Form III. No peaks relating to the monohydrate form are present after 4 hr at 4% RH, 40 °C. Coupling these results with the DVS work presented in **Sections 5.3.2.1.1** and **5.3.2.1.3**, the lack of stability of the monohydrate form low humidity conditions (0%/4% RH) was confirmed.

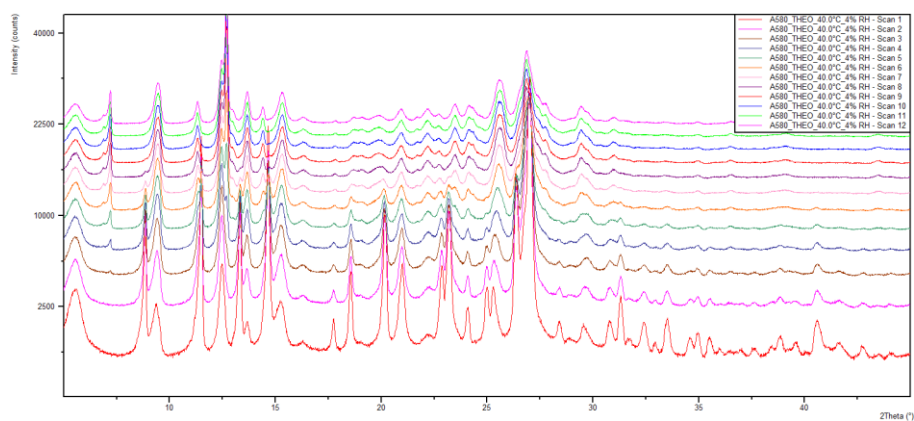


Figure 5.60: Non-ambient XRPD overlay of THEO monohydrate being held at 4% RH, 40 °C for 4 hr. (Bottom to top - first scan to last scan).

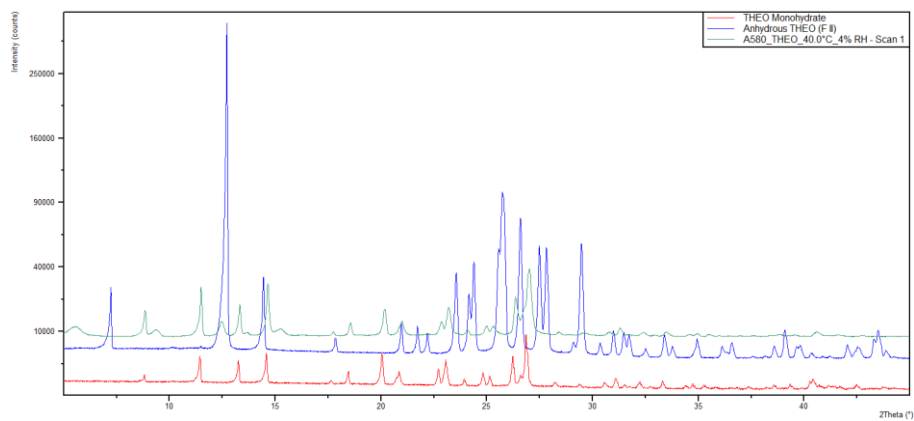


Figure 5.61: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the first scan of THEO monohydrate being held at 4% RH, 40 °C.

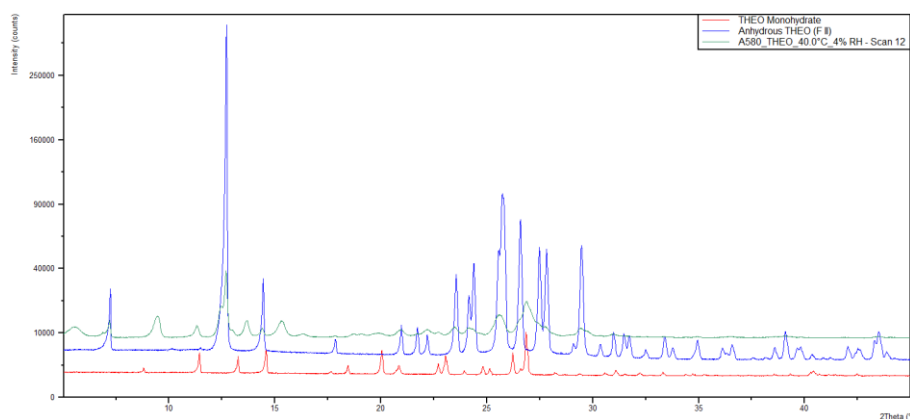


Figure 5.62: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the last scan of THEO monohydrate after being held at 4% RH, 40 °C for 4hr.

5.3.2.1.4.4.1.2. 40% RH, 40 °C for 2 hr, switching to 4% RH, 40 °C for 2hr

Figure 5.63 shows the XRPD overlay of scans throughout the experiment, with the change in % RH (from 40 to 4% RH) made clear through the extra space after the first four scans. The gradual disappearance of the characteristic peaks of the monohydrate was only evident after the % RH value was reduced to 4% RH but the growth of new peaks is seen after the first scan onwards. **Figure 5.64** confirmed the presence of the monohydrate at the start of the experiment. The first scan at 4% RH showed many of the characteristic peaks of THEO monohydrate but with a decrease in intensity. From the second scan at 4% RH, many of peaks of the monohydrate were lost. The final scan, **Figure 5.65**, compared well to the metastable anhydrous THEO Form III, ^[90] except for one peak 8.9° 2θ which corresponds to a peak from the original monohydrate form. DVS work at the same conditions (**Sections 5.3.2.1.1** and **5.3.2.1.3**) confirmed that the monohydrate can remain stable at 40% RH and 40 °C, after losing the excess moisture, until the lower % RH step is implemented. Differences in end point samples to that of the post DVS samples could be due to the non-ambient XRPD

chamber being unable to reach 0% RH. Analyses at the higher value of 4% RH may have a different effect.

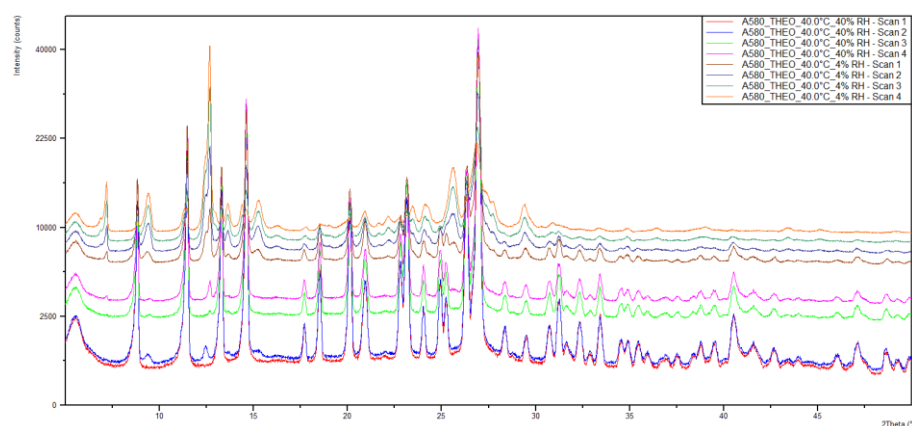


Figure 5.63: Non-ambient XRPD overlay of THEO monohydrate being held at 40% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr. (Bottom to top - first scan to last scan).

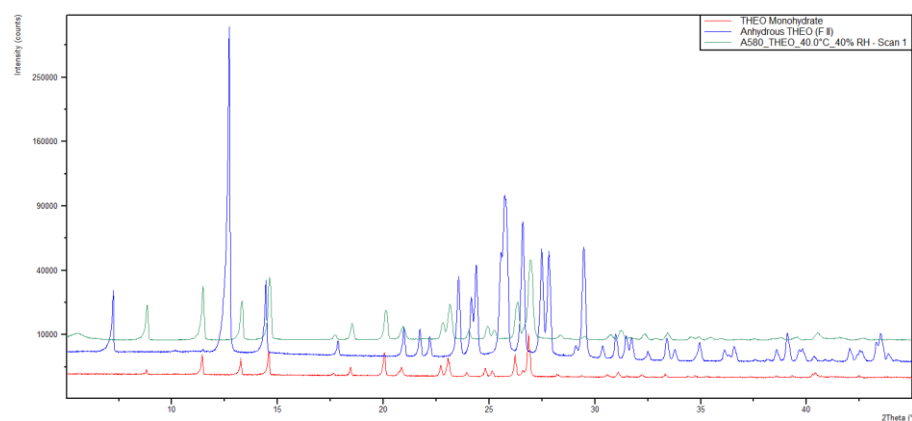


Figure 5.64: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the first scan of THEO monohydrate being held at 40% RH, 40 °C.

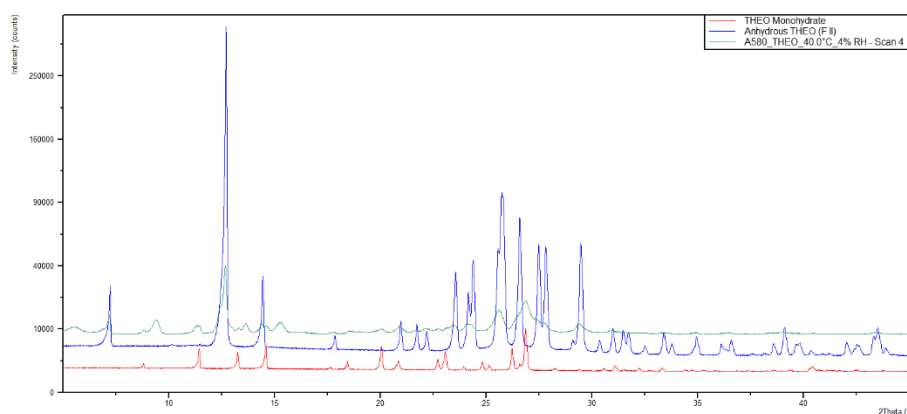


Figure 5.65: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the last scan of THEO monohydrate after being held at 40% RH, 40 °C for 2hr to 4% RH, 40 °C for 2hr.

5.3.2.1.4.4.1.3. 80% RH, 40 °C for 2 hr, switching to 4% RH, 40 °C for 2hr

Figure 5.66 shows the XRPD overlay of scans throughout the experiment, with the change in % RH (from 80 to 4% RH) made clear through the extra space after the first four scans. The gradual disappearance of the characteristic peaks of the monohydrate was only evident after the % RH value was reduced to 4% RH. The monohydrate form remained stable throughout the 80% RH step of the method. **Figure 5.67** confirmed the presence of the monohydrate at the start of the experiment. From the second scan at 4% RH, many of peaks of the monohydrate were lost and the growth of peaks relating to an anhydrous form became evident. The final scan (**Figure 5.68**) compares well to the metastable anhydrous THEO Form III, ^[90] but similar to the sample in the previous section, the end point sample contained one peak at 8.9° 2θ which related to the original monohydrate form. DVS work at the same conditions (**Sections 5.3.2.1.1** and **5.3.2.1.3**) confirmed that the monohydrate can remain stable at 80% RH and 40 °C, until the % RH is reduced. **Figure 5.69** shows a comparison of each of the end point samples from **Sections 5.3.2.1.4.4.1.1, 5.3.2.1.4.4.1.2**

and 5.3.2.1.4.4.1.3, with all three traces overlaying well, except for the monohydrate peak at $8.9^\circ 2\theta$ which was only present in the last two samples (held at 40% RH or 80% RH initially).

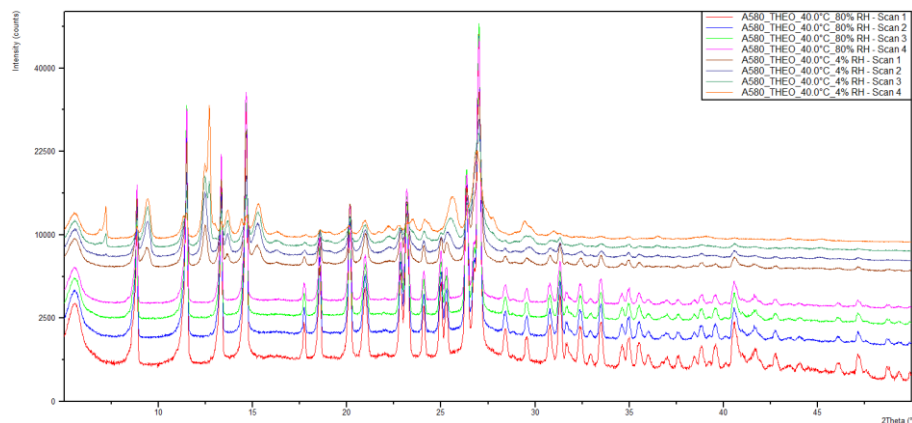


Figure 5.66: Non-ambient XRPD overlay of THEO monohydrate being held at 80% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr. (Bottom to top - first scan to last scan).

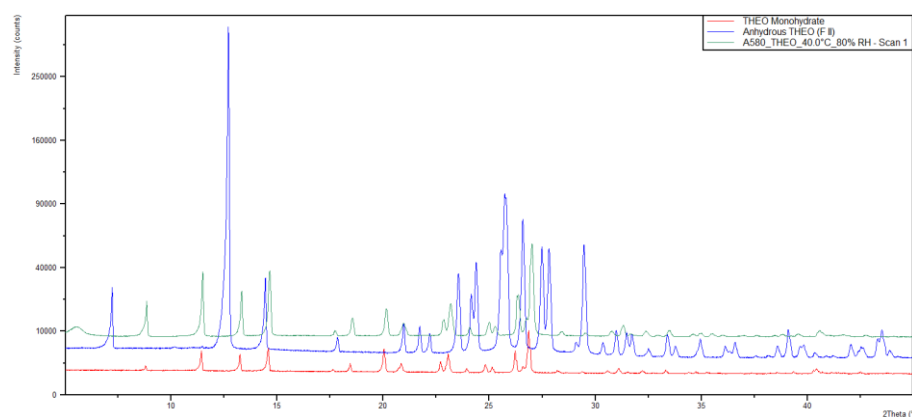


Figure 5.67: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the first scan of THEO monohydrate being held at 80% RH, 40 °C.

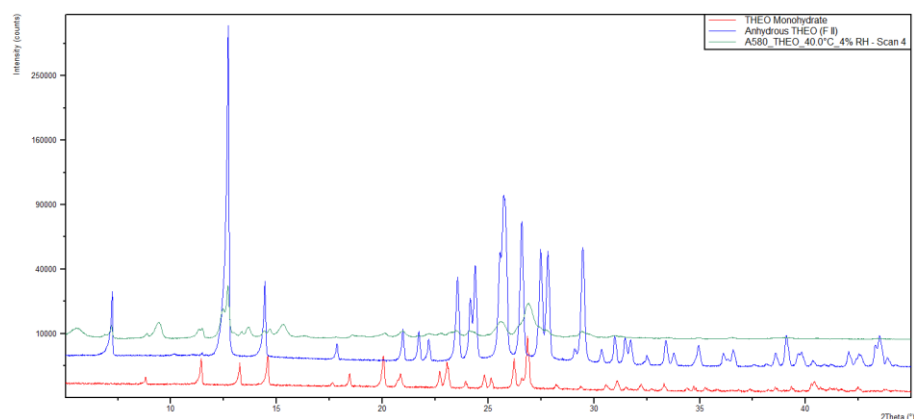


Figure 5.68: Non-ambient XRPD overlay of THEO monohydrate, anhydrous THEO Form II and the last scan of THEO monohydrate after being held at 80% RH, 40 °C for 2 hr to 4% RH, 40 °C for 2 hr.

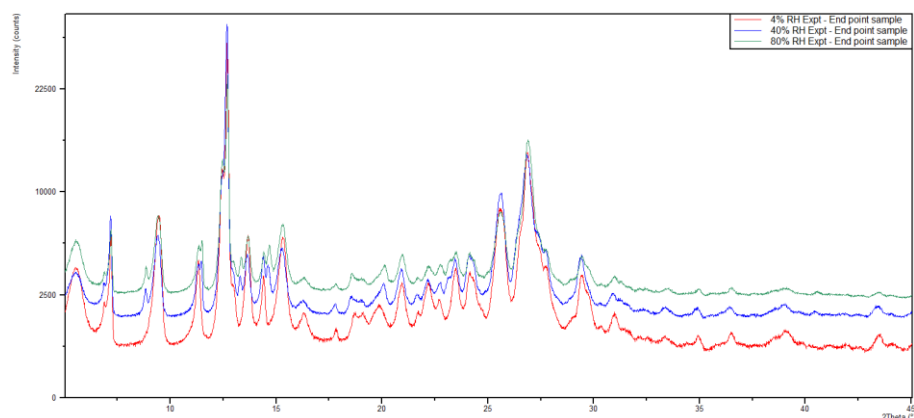


Figure 5.69: Non-ambient XRPD overlay of end point samples for THEO.

5.3.2.2. Effects of temperature on wet THEO monohydrate at 30 and 40 °C

To examine the effects of temperature, experiments were carried out at 30 and 40 °C, with increasing RH levels of 0, 10, 20, 40, 60 and 80% RH. Each experiment involves the specific % RH being held for 120 min, after which the RH level is dropped to 0% RH to fully remove all moisture from the sample.

For THEO monohydrate, a 600 min 0% RH step was used across all samples analysed at 30 °C and a 120 min 0% RH step for 40 °C samples. **Figure 5.70**, below, shows the change in moisture content

over time for each sample at both 30 and 40 °C. This graph includes the suggested regions of removal of loosely bound moisture (1), periods of hydrate stability (2) and the dehydration/loss of water of hydration (3).

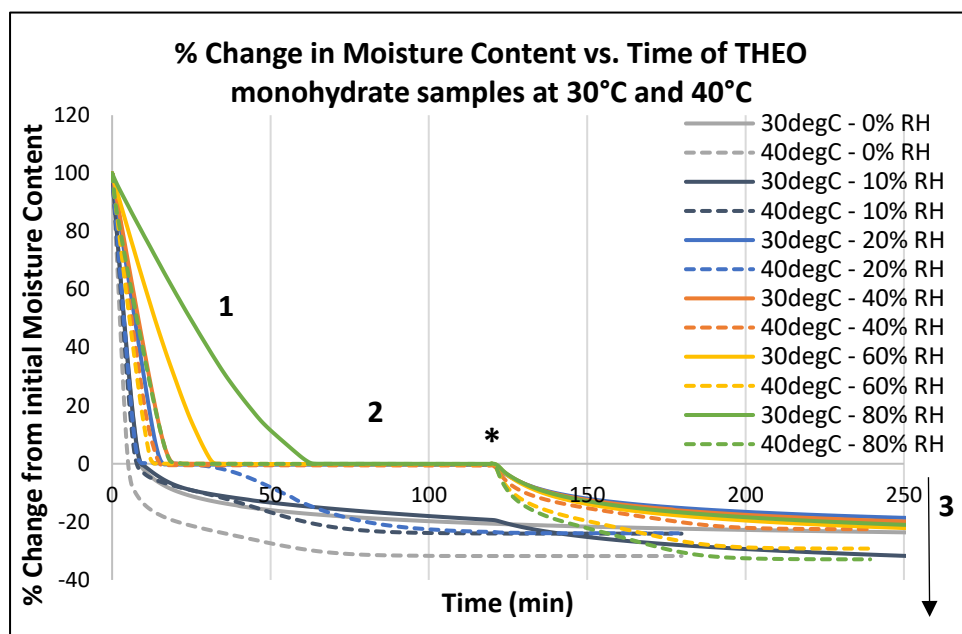


Figure 5.70: % Change in Moisture Content vs. time for THEO monohydrate at 30 and 40 °C with increasing levels of relative humidity.

* denotes the start of the 0% RH step.

5.3.2.2.1. Effects of temperature on the drying profile of THEO monohydrate

As previously discussed in **Section 5.3.1.1.1**, moisture content values >0% are the excess, loosely bound moisture. To examine the drying profiles and the effects caused by temperature differences, **Figure 5.71** provides a zoomed in section of **Figure 5.70**, showing Region 1, the drying profiles of each sample.

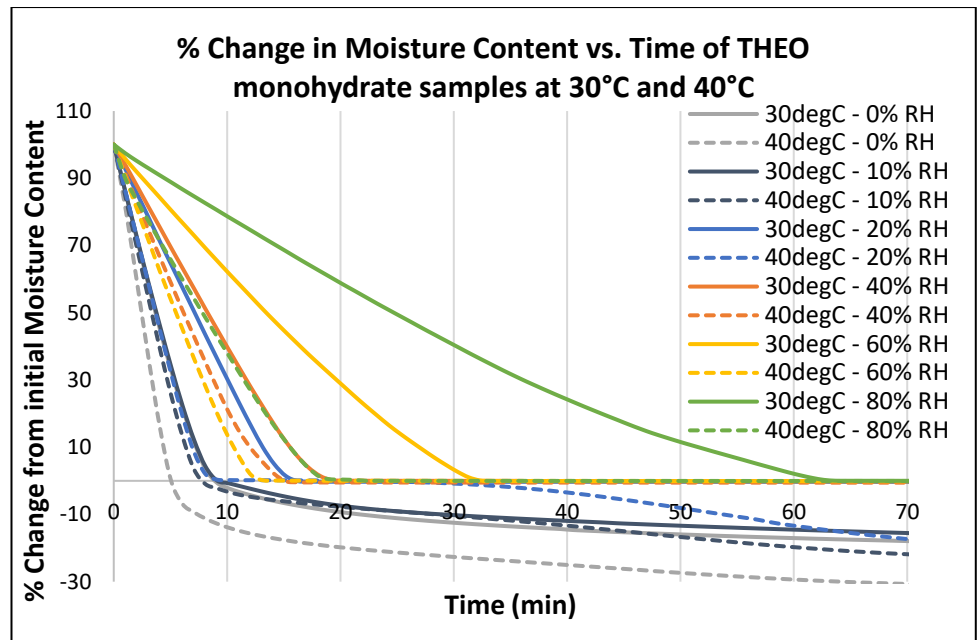


Figure 5.71: % Change in Moisture content vs. time for THEO monohydrate at 30 and 40°C and varying %RH, showing the drying profiles.

Higher temperatures result in shorter drying times and at 40% RH and above, dehydration only begins when the 0% RH step begins. Dry THEO monohydrate cannot be obtained at either temperature, below 20% RH.

Data shows that at 0% RH, drying occurs very quickly at both temperatures, in less than 10 min. At 40 °C, the point of inflection occurred in the region which was proposed to be of dehydration/loss of bound water. It is possible that drying occurred so quickly that for a period, both drying of loosely bound and removal of water of hydration was happening in parallel. At 30 °C, the point of inflection can be seen on the x axis, suggesting the dry hydrate was present before the more tightly bound moisture began to be removed and the drying rate shifted slightly. At 10% RH a point of inflection on the x-axis is seen for both temperature samples, suggesting the dry monohydrate was present for a very short time prior to the removal of this water of hydration. Once

again, drying occurred quickly at both 30 and 40 °C with drying times being 9 min and 8 min respectively. At 20% RH, increasing the temperature from 30 °C to 40 °C saw the drying time reduce by approximately 50%, from 16 min at 30 °C to 8 min at the higher temperature. Drying of THEO monohydrate at these low levels of RH, results in only short periods of hydrate stability making the boundaries for obtaining the dry monohydrate difficult to work within. At 40% RH, both samples took less than 20 min to dry. At 60% RH there is a greater difference in drying times. The effect of the temperature increase is clear, with the 40 °C sample losing its excess, loosely bound moisture after 13 min, while at 30 °C, drying took approximately 32 min. At the highest RH level of 80%RH, drying times at 40 °C were 19 min and at 30 °C, drying times increased to 62 min.

By looking at the traditional drying rate curves of all samples (**Figure 5.72** below), higher drying rates are seen in all samples analysed at 40 °C. This further backs up the conclusion that across all levels of relative humidity, the higher the temperature, the shorter the drying times.

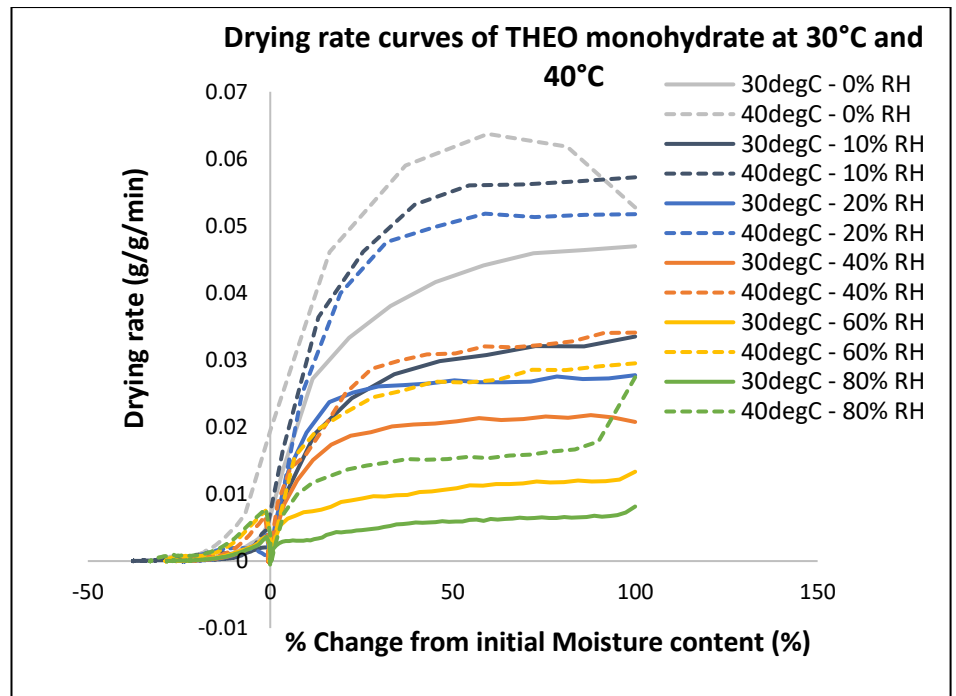


Figure 5.72: Drying curves for THEO monohydrate at 30 and 40 °C with increasing levels of relative humidity.

5.3.2.2.2. Effects of temperature on the hydrate stability of THEO monohydrate

Figure 5.73 shows a zoomed in portion of the graph shown in **Figure 5.70**, depicting the periods of hydrate stability, Region 2 (if any), after losing excess, loosely bound moisture.

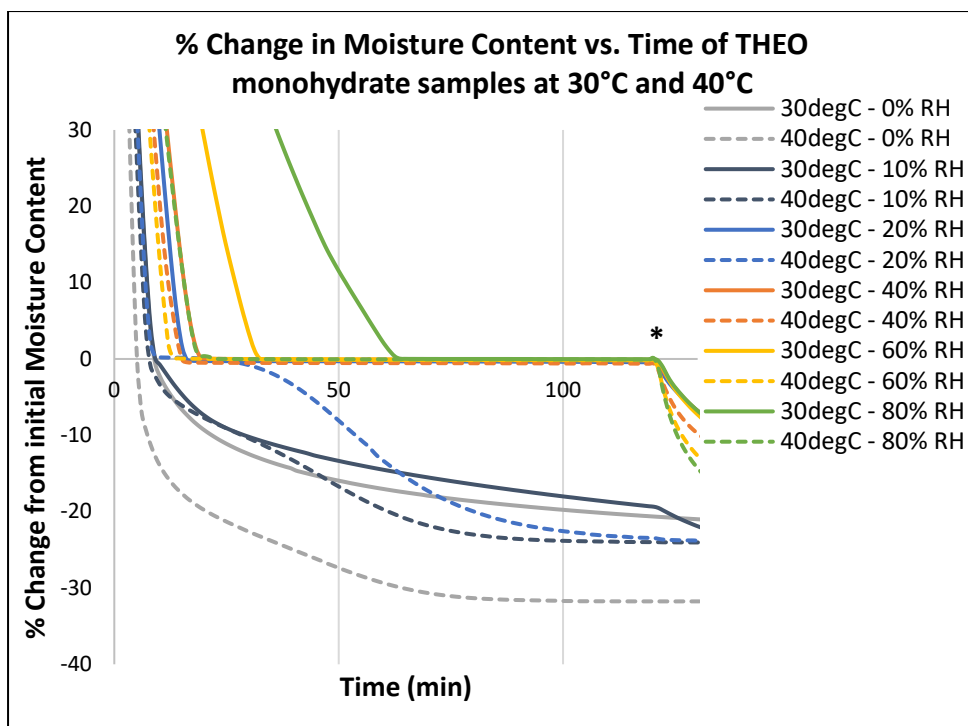


Figure 5.73: % Change in Moisture Content vs. time for THEO monohydrate at 30 °C and 40 °C and varying %RH, showing periods of hydrate stability where conditions allow. * denotes the start of the 0% RH step.

At 0 and 10% RH, there is no period of hydrate stability at either temperature. At these conditions, at both temperatures, it is not possible to obtain the dry monohydrate sample post drying. Changes based on temperature begin to be seen at 20% RH. Hydrate stability can be seen in both samples, but they differ greatly. At 30 °C, the moisture content of 0% is reached at 17 min and holds up to ~70 min after which there is a small but gradual reduction in moisture content until the 0% RH step begins. The obvious loss of the remaining moisture can be seen. At 40 °C this period of hydrate stability is reduced, lasting approximately 10 mins before the moisture content starts to reduce. At this higher temperature, all moisture is removed from the sample prior to the 0% RH step starting.

At RH levels of 40% and above, hydrate stability can be seen across all samples, at both temperatures. After drying, hydrate stability lasted for the remainder of each initial % RH step and was only disrupted when the 0% RH step began.

5.3.2.2.3. Effects of temperature on dehydration profile after drying of THEO monohydrate

Figure 5.74 shows a zoomed in portion of the graph shown in **Figure 5.70**, depicting the tightly bound moisture, water of hydration being removed.

Overall, dehydration at the lower temperature was slower compared to dehydration at 40 °C, even during the 0% RH step. For samples analysed at 0 and 10% RH, drying of loosely bound moisture is quickly followed by dehydration. At 10% RH, the effects of temperature became more evident. At the higher temperature of 40 °C, all moisture is fully removed prior to the start of the 0% RH step, whereas at 30 °C, drying was followed by a dehydration phase, but moisture loss occurred more slowly. Moisture was still present at 120 min, which was then removed with the start of the 0% RH step. At 20% RH, the effects of temperature were more pronounced. At 40 °C, after a short time of hydrate stability, dehydration began and fully removed the water of hydration before the 120 min time point. The 30 °C sample at 20% RH showed hydrate stability after the initial drying phase (to remove loosely bound moisture) until the 0% RH step began. At the higher levels of RH, 40, 60 and 80% RH, dehydration only began when conditions changed with the 0% RH step. The effects of the temperatures are seen in the time taken to reach stability in the % M.C post dehydration. At 40 °C, a stable % M.C. is seen at the 200 min time point, whereas for the 30 °C samples, stability of % M.C. is seen at

approximately the 600 min mark. Reducing the temperature resulted in slower dehydration.

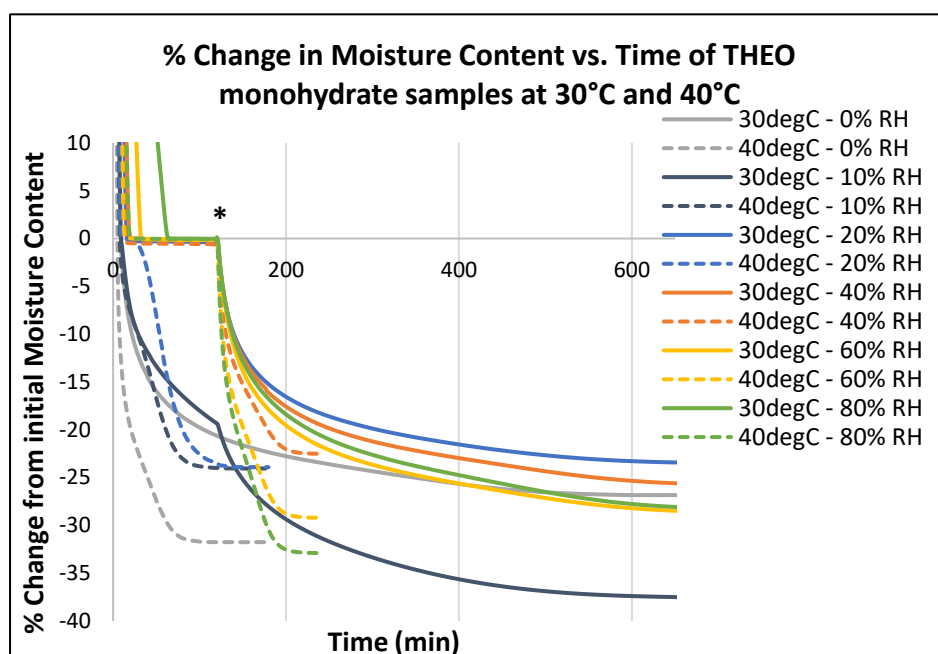


Figure 5.74: % Change in Moisture Content vs. time for THEO monohydrate, showing the dehydration profiles at 30 °C and 40 °C and varying %RH. *denotes the start of the 0% RH step.

5.3.2.3. Effects of particle size on the drying on wet THEO monohydrate

To investigate the effects of particle size on the drying, stability, and dehydration of THEO monohydrate, portions of ‘wet’ THEO monohydrate were generated of increasing particle size. The samples discussed here are different to those of differing PSD discussed in **Section 3.3.4.3**. The batch of THEO monohydrate used throughout the drying study via the DVS described above was generated using the standard method detailed in **Section 2.3.1.6**. These crystals were considered ‘Medium’ in size. As the standard method involved a cooling crystallisation, a change in cooling rates was used to generate particles of different sizes. See **Sections 2.3.1.7.1** and **2.3.1.7.3** for more details.

PSD data was collected for each sample using a Malvern 3000 (Section 2.4.6). Table 5.5 shows the d10, d50 and d90 averages across the six data points for each batch of THEO monohydrate. Across each sample and across each portion of particles, PSD values increased as desired. The PSDs for each data set (Figure 5.75) shows a shift to the right (larger particle size) for Small to Medium to Large, confirming the presence of larger crystals.

Table 5.5: PSD data for the Small, Medium and Large THEO monohydrate samples.

Particle size distribution parameters (average $\pm$ std. deviation)			
Sample	D10 (μm)	D50 (μm)	D90 (μm)
Small	12.20 $\pm$ 0.93	36.90 $\pm$ 2.63	153.00 $\pm$ 12.20
Medium	13.80 $\pm$ 0.06	47.20 $\pm$ 0.29	196.00 $\pm$ 10.30
Large	20.90 $\pm$ 1.59	69.60 $\pm$ 5.51	204.00 $\pm$ 12.80

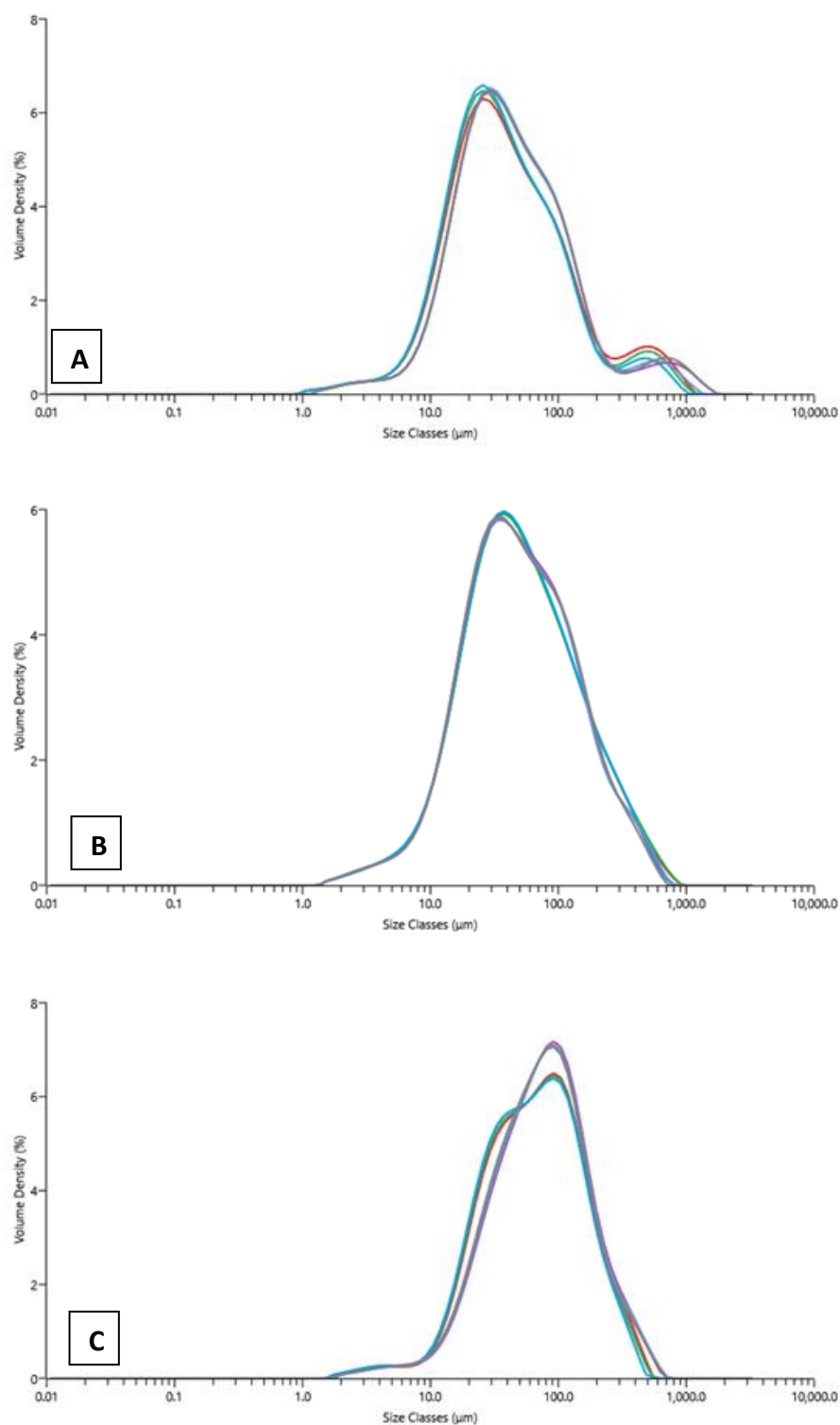


Figure 5.75: PSD overlay of all **(A)** Small, **(B)** Medium, and **(C)** Large THEO monohydrate samples.

Once PSD data confirmed the samples had increasing PSD values, samples were analysed in the DVS at 40% RH and 40 °C for 120 min, followed by the 0% RH step. **Figure 5.76** shows the full profiles.

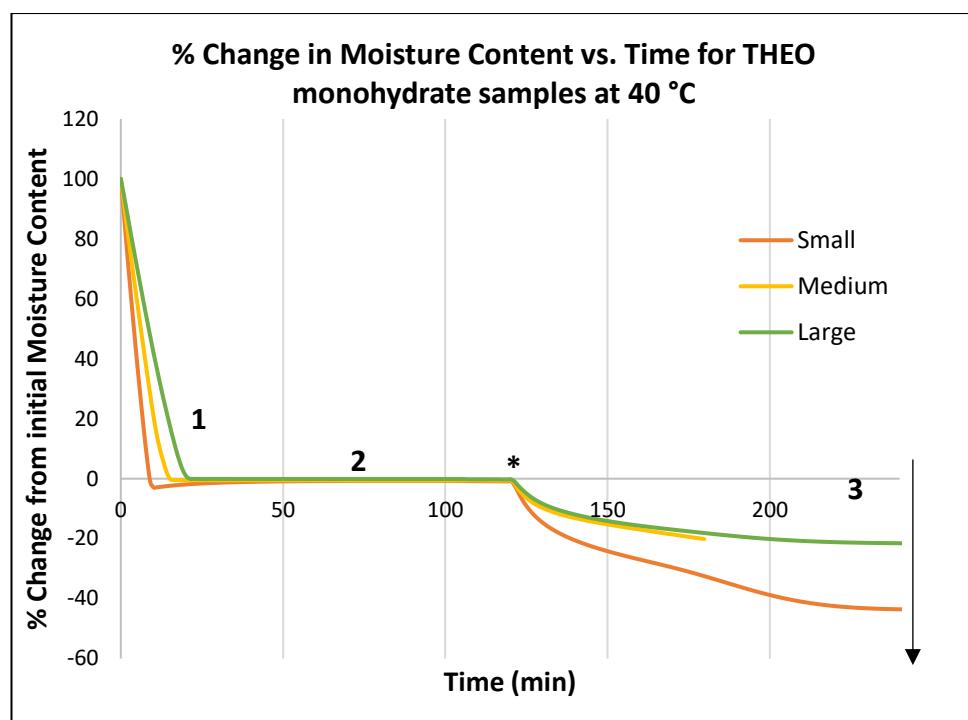


Figure 5.76: % Change in Moisture Content vs. time for Small, Medium, and Large sized THEO monohydrate crystals at 40% RH and 40 °C. * denotes the start of the 0% RH step.

5.3.2.3.1. Effects of particle size on the drying profile of THEO monohydrate

To examine the drying profiles more closely, **Figure 5.77** shows a zoomed in section of **Figure 5.76** showing only the drying curves, Region 1, of the three samples.

Results shown below are the opposite of what was seen in **Section 5.3.1.3.1** for CBZ. The Large CBZ dihydrate particles dried fastest, which was unexpected. Normally, powders comprised of smaller particles, with a larger surface area, would be expected to have faster drying times and this is the case for THEO monohydrate samples. Drying times increased with an increase in particle size (reduction in surface area from which drying can occur) across each sample set. Drying times increased by 5 min in each instance of moving from Small to Medium and Medium to Large crystals.

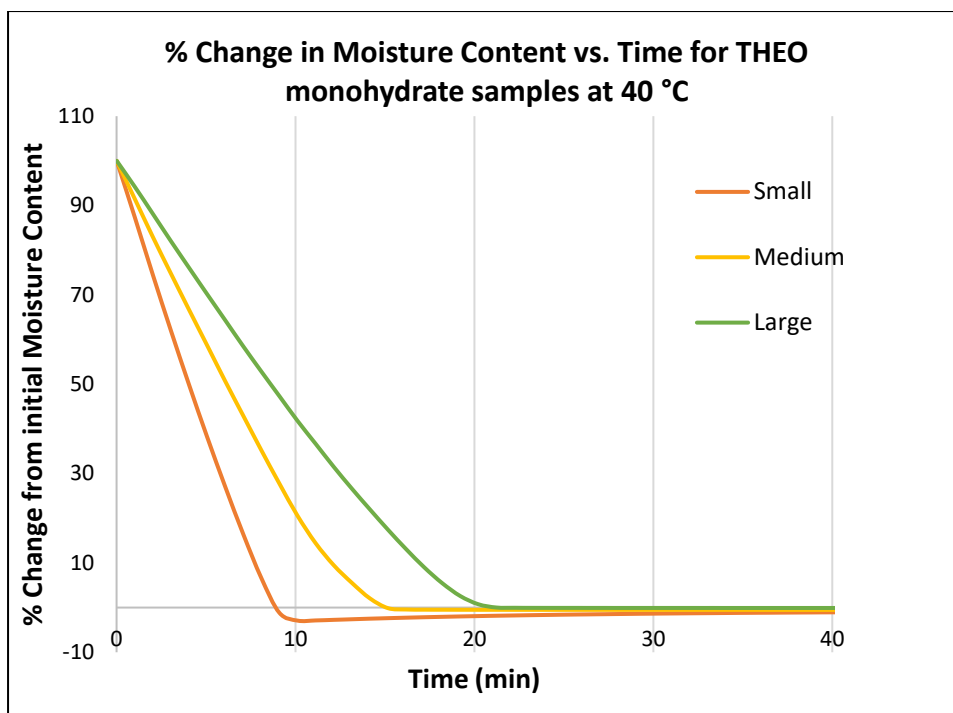


Figure 5.77: % Change in Moisture content vs. time for Small, Medium, and Large sized THEO monohydrate crystals showing the drying profiles at 40% RH and 40 °C. * denotes the start of the 0% RH step.

5.3.2.3.2. Effects of particle size on the hydrate stability of THEO monohydrate

The dry monohydrate is evident and stable, post drying across all three samples. The hydrated forms remain in place until the start of the 0% RH step, (**Figure 5.78**). Examining the drying curve transition into the stability period of the curve, the Small particle sample shows very fast drying. This fast drying goes beyond only loosely bound moisture and negative values of % M.C. are seen, which equates to dehydration. However, during the time at 40% RH, the sample appears to take up some moisture from the environment and find a stable % M.C. at ~50 min which correspond to the dry monohydrate. In **CHAPTER 3**, the multiple cycle method on the DVS proved that the THEO monohydrate lattice structure was strong enough to withstand numerous sorption and desorption cycles and this is what is evident here, albeit a minor version of that.

When held in an appropriately wet environment, THEO monohydrate will incorporate moisture up to the stoichiometric value for the monohydrate. Stability of the Small THEO monohydrate crystals were seen from 50 min to the 120 min time point, where the 0% RH step began, and dehydration took place.

Hydrate stability for Medium and Large crystals was seen immediately post drying and was stable for the remainder of the time at the 40% RH conditions. The start of the 0% RH step then induced dehydration.

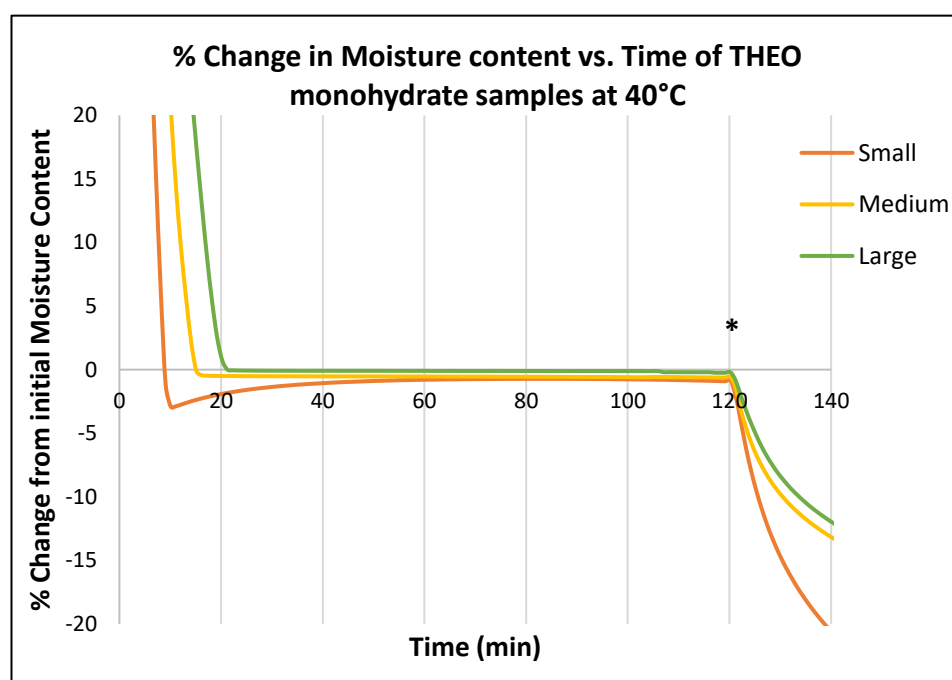


Figure 5.78: % Change in Moisture content vs. time for Small, Medium, and Large sized THEO monohydrate crystals at 40% RH and 40 °C, showing periods of hydrate stability, where % moisture content equals 0%. * denotes the start of the 0% RH step.

5.3.2.3.3. Effects of particle size on the dehydration profile after drying of THEO monohydrate

Dehydration of all three samples did not begin until the 0% RH step. Once the % RH drops, the initial rate of dehydration is greatest in the Small particle sample. However, the time taken for stability to be found in the % M.C. values of each sample took ~100 min. As the dehydration times were similar, it was not affected by differences in PSD across the samples.

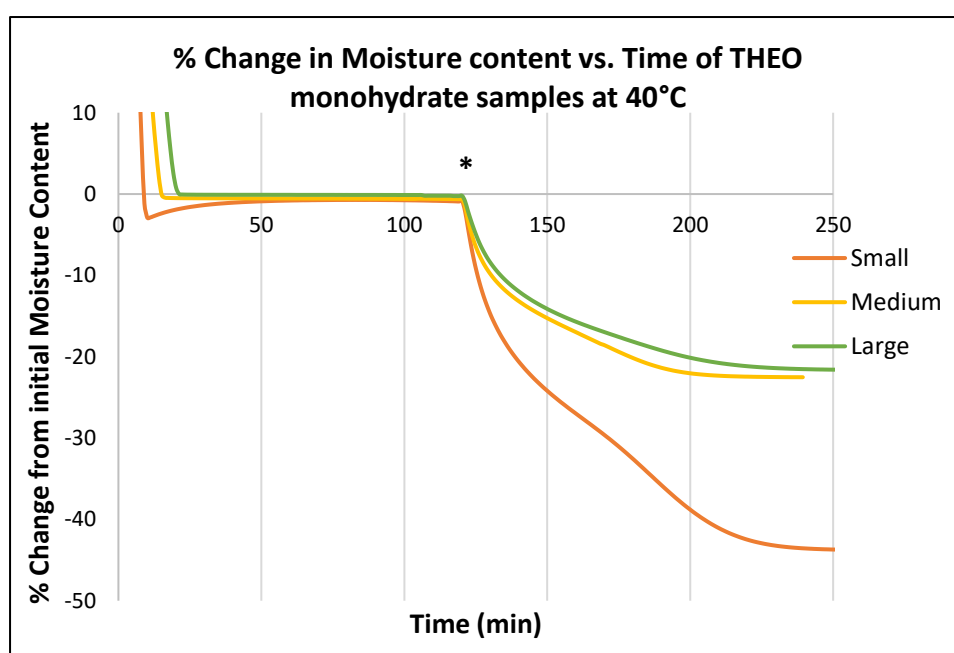


Figure 5.79: % Change in Moisture content vs. time for Small, Medium, and Large sized THEO monohydrate crystals at 40% RH and 40 °C, showing the dehydration profiles. * denotes the start of the 0% RH step.

5.3.2.4. Investigating hydrate stability

In this section, hydrate stability under 12 hr holds was investigated. Experiments were carried out at 40 °C and at 40% RH and 60% RH. 40% RH was chosen as the starting point for this study as it was the first set of conditions which showed significant stability of the hydrated form.

5.3.2.4.1. Effects of 12 hours hold at 40 and 60% RH

For this work, the initial step was held for 12 hr and then followed by the 0% RH step.

Figure 5.80 shows the results, where the differences due to the levels of % RH are clear. At 40% RH, we see the initial slope for the drying of loosely bound moisture, which is steeper than the 60% RH sample and then stability of the dry monohydrate. This stability begins to fall away at approximately 300 min and dehydration slowly begins. The rate of dehydration is clearly impacted by the 40% RH environment, however it continues slowly until 720 min. Here the 0% RH step begins, and its effects are obvious, with dehydration then taking ~80 min to remove the remaining moisture. At 60% RH, post drying, the dry monohydrate remains stable for the remainder of time in the 720 min step. Changes are only seen when dehydration is once again induced by the 0% RH step. Dehydration of the full amount of water of hydration also took ~80 min here too.

Drying at 40 °C and either 40 or 60% RH look to be advantageous drying protocols for THEO monohydrate, as both conditions dry the sample quickly and provide the dry monohydrate, which is stable for an extended period, allowing for it to be obtained in its desired form.

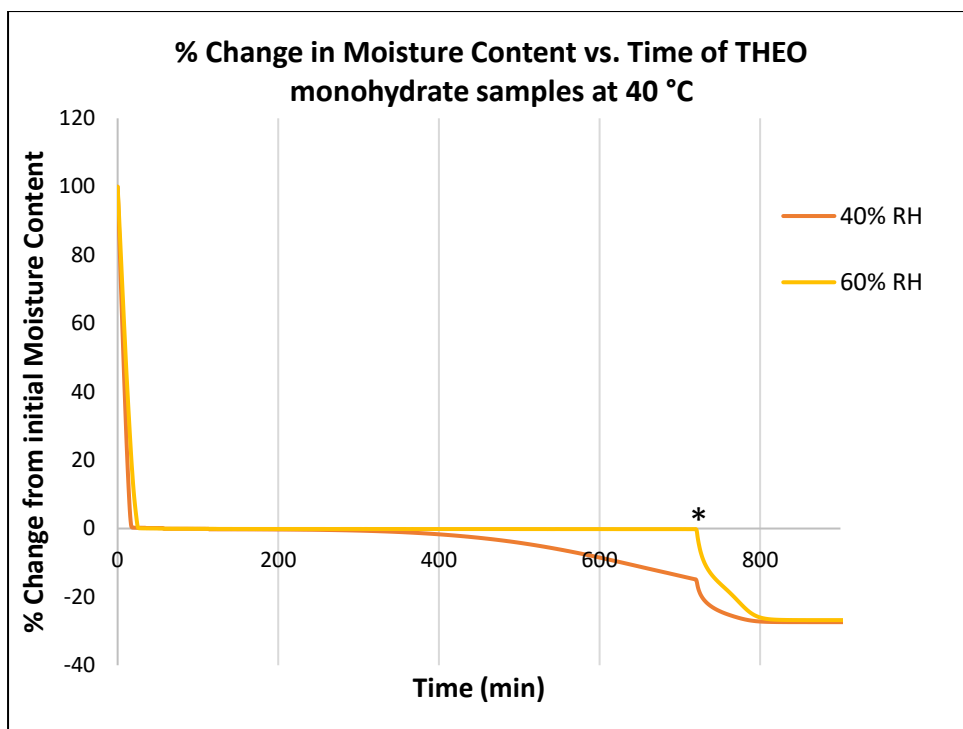


Figure 5.80: % Change in Moisture Content vs. time for THEO monohydrate samples with 12 hr hold times at specific % RH. * denotes the start of the 0% RH step.

5.3.2.4.1.1. Solid-state analysis of post DVS samples

As previously indicated, post DVS samples range from 10-25 mg so choosing the most beneficial types of analysis was important. To examine the effects of long hold times samples were analysed by XRPD.

5.3.2.4.1.1.1. XRPD Analysis

Results of post DVS samples saw them overlaying well with the anhydrous starting material (**Figure 5.81**). As discussed previously (**Section 3.3.1.2**), THEO monohydrate can withstand numerous cycles of sorption in high relative humidity environments and desorption in low relative humidity environments, with the lattice remaining intact as water enters and exits through its channels. Based on this, seeing the XRPD traces move from the hydrated form

to the starting material would be expected. The reduced resolution of the 60% RH sample is due to the sample preparation. All efforts were made to optimise sample preparation and to concentrate the sample into the middle of the holder. Resolution shown here, is the best that could be achieved with these samples.

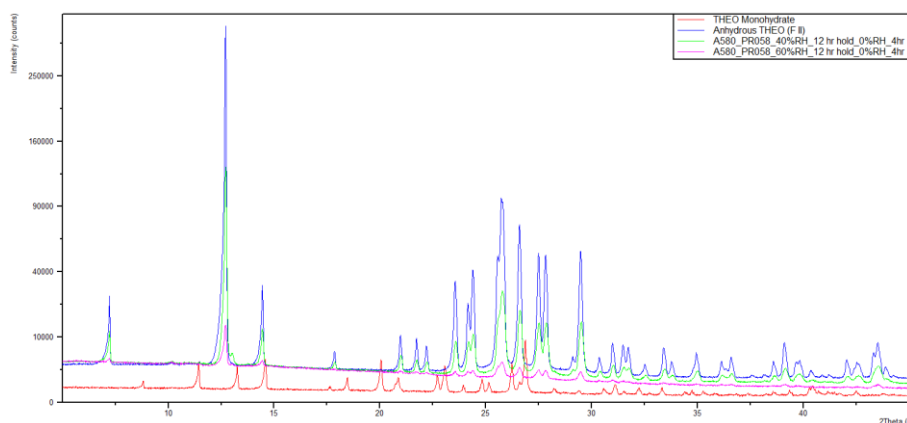


Figure 5.81: XRPD overlay of THEO monohydrate (red), anhydrous Form III starting material (blue), post DVS at 40% RH for 12 hr, 0% RH for 4 hr (green) and post DVS at 60% RH for 12 hr, 0% RH for 4 hr (pink).

5.3.2.5. Investigating the effects of increased sample size

In this section, the effect of increased cake depth of drying time was investigated. Experiments were carried out at 40 °C, 40% RH for 2 hr, followed by 40 °C, 0% RH for 2hr. Samples were prepared in the same way as detailed in **Section 2.8.1** but increasing the sample weight to ~40-60 mg. Other DVS experiments had average sample weights of 15-20mg. Sample preparation also involved ensuring the same flat surface of the pan was covered but with the increased sample weight/depth. By increasing sample amount and overlaying the results with the previously analysed sample at the same conditions, it was found that drying time was not adversely affected by an increased sample amount or cake depth, as shown in **Figure 5.82**. Sample sizes within the DVS are considered very small when compared to sample cakes dried in lab ovens. Small

sample cakes, such as those examined from the DVS, might not be the ideal cases for studying the differences in mass transfer during drying. Despite this, when looking at DVS data on its own it is beneficial information to have that cake depth had only a minimal effect on drying times. When compared to the same analysis of CBZ dihydrate (**Figure 5.43**), very different results were seen. While THEO monohydrate showed a small difference in drying time with an increased sample amount, CBZ dihydrate saw its drying time more than double. This could be due to differences in cake depth over just sample weight alone. The wet CBZ dihydrate tended to clump more easily and had a larger cake depth value than the more compact cake of THEO monohydrate. A larger cake depth would result in a greater migration distance for moisture to travel to be removed. As the work discussed in **Sections 5.3.1.1.1** and **5.3.2.1.1** detailed the shorter drying times for THEO monohydrate samples, the difference seen here is likely due to sample amount/cake depth over crystal packing.

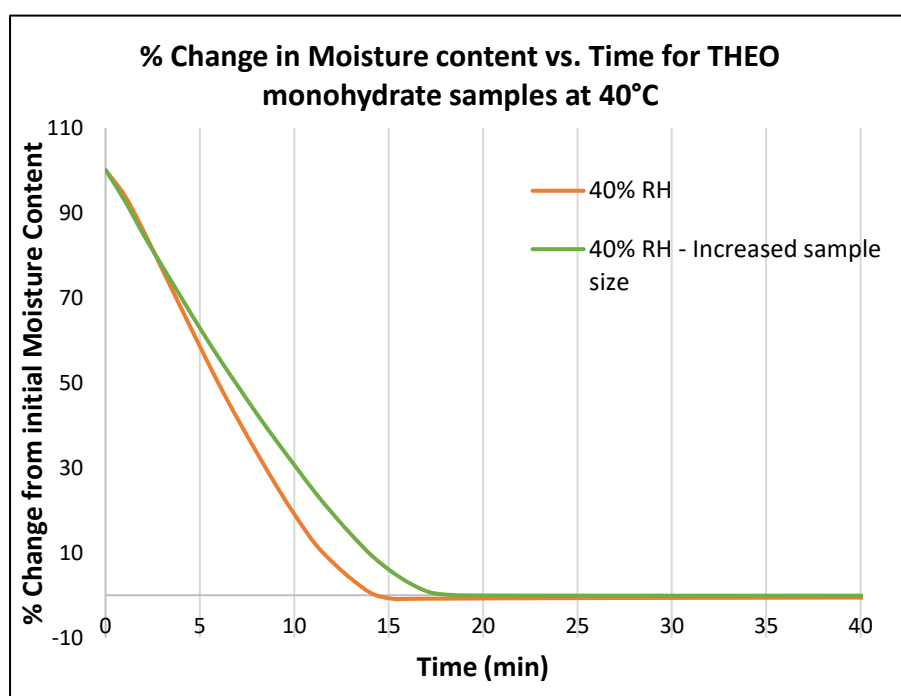


Figure 5.82: Drying curves of THEO monohydrate samples, comparing the drying times with increased sample weight.

5.4. Chapter Conclusions

5.4.1. CBZ Dihydrate

For CBZ dihydrate, results showed that an increase in % RH gave rise to increased drying times. Drying rates were shown to decrease with increasing levels of relative humidity also. An increase in temperature resulted in shorter drying times. Both were expected results. Periods of hydrate stability (i.e., where the dried hydrate was retained without dehydration) were found in environments of 40% RH and above. Investigation into hydrate stability determined that at 40% RH, the stability waned after ~150 min. However, at 60% RH, dry dihydrate remained stable for the entire 12 hr hold time and was only altered by the start of a 0% RH step. When examining the dehydration profiles, at low % RH, the loss of water of hydration took place mostly within the low RH environment itself (i.e., before the later imposition of a 0% RH condition). From 40% RH and above, the 0% RH step induced dehydration.

Investigating the effects of PSD, it was found that the Large crystals had a quicker drying time, which was unexpected. Powders comprised of smaller particles with a larger surface area are expected to have quicker drying times. At 40% RH, 40 °C hydrate stability was only seen with the Medium crystals. The other samples saw very small amounts of dehydration during the initial 40% RH, 40 °C step. Dehydration during the 0% RH step was similar for Small and Medium particles which was expected based on similar d10 and d50 values. The Large particles had the longest dehydration time and is likely due to the water of hydration taking longer to move through the channels of the larger dihydrate crystals. Increasing the sample amount in the DVS, more than doubled the drying time. The dihydrate tended to clump when being kept damp so the cake depth of this sample was significantly

more than the standardised DVS sample, and this increase in migration distance for the moisture to travel would play a big role in the drying time.

5.4.2. THEO Monohydrate

An increase in % RH caused an increase in drying times, except in the first set of samples analysed, which saw one of the three samples analysed at 40% RH take ~2-3 min longer to dry than the 60% RH sample. Drying times across all samples were short; even at 80% RH, drying was completed within 20 min. Drying rates were shown to decrease with increasing levels of relative humidity, which was an expected outcome. Hydrate stability was only seen in samples dried at 40% RH and above. Drying rates of samples held at $\geq 40\%$ RH showed that, despite the dry monohydrate being stable in environments of increasing RH values, each experienced the same rate of dehydration once the 0% RH step started. Investigations into hydrate stability found that at 40% RH, that stability was lost after ~300 min. However, at 60% RH, dry monohydrate remained stable for the 12 hr hold period and was only impacted once the 0% RH step had begun. Results proved that an increase in temperature caused shorter drying times across each % RH level. When examining hydrate stability, after removal of the loosely bound moisture, findings showed that in environments of $\geq 40\%$ RH, temperature did not affect stability of the monohydrate when working at temperatures of 30 °C or 40 °C. Below 40% RH, the higher temperature reduced the monohydrate stability.

Investigating the effects of PSD, it was found that drying times increased with increased particle size, which was a much more expected result, based on surface areas exposed to the drying environment, than was found for the CBZ samples. THEO monohydrate samples showed that smaller particles, with large

surface area available for drying gave quicker drying times. Increasing the sample amount, increased the drying time by only ~3 min. The THEO monohydrate is a less clumped damp cake compared to CBZ dihydrate and, while sample amount was increased, the cake depth was less than that of the CBZ dihydrate “larger” sample, which could be the reason behind the differences in drying times for them both.

Overall, the DVS has proven to be a highly useful, accurate and novel approach for gathering drying data of wet cakes. Using this data, insights into drying times and kinetics can be examined and ultimately, the time point where the loosely bound moisture was removed, prior to the water of hydration being affected was found. Due to the success, work then began on the development of a predictive drying model which will be discussed in the next chapter.

CHAPTER 6. Predictive drying model for convective drying – Model development and constant rate period validation

6.1. Abstract

In this chapter, DVS experimental data was filtered to obtain Moisture Content (X) and experimental time (θ_{exp}) data. Using these values, the data was used to predict the Critical Moisture Content (X_C) and convective heat transfer coefficient (h_C) for data sets across a range of % RH levels and temperatures. Assumptions from the DVS drying studies included:

- Critical Moisture Content (X_C) is independent of drying gas properties
- Convective heat transfer coefficient (h_C) is a function of gas flowrate only.

With these assumptions, for oven drying and its predictive model, the X_C remains the same whereas the h_C must be scaled up to match the oven conditions. The scale of the gas flowrate in the oven is compared to that of the DVS. The final model detailed in this chapter is the most recent version with previous iterations being updated as new issues were considered and overcome. In the latest model, X_C and h_C are predicted for different sets of drying conditions as X_C was found to vary depending on % RH. Earlier version of the model only incorporated the %RH value set outside the oven, this resulted in consistent underpredictions of drying times. A later iteration of the model involved a new calculation for the % RH being experienced inside the oven, based on what % RH was set outside. The oven pressure and the resulting vapour pressure being generated (calculated using the Antoine equation) were also incorporated. Understanding the difference between

setting a % RH on the outside and what % RH level is being experienced by the sample on the inside proved to be an important aspect in translating what was learned through the Drying Studies via the DVS to the oven drying predictions.

In the final version of the model, the Antoine equation is used in combination with a new calculation for the wet bulb temperature (T_w). The Antoine equation is used to calculate the % RH inside the oven, based on the oven temperature, pressure and % RH set on the outside. This new value is used in an equation that relates the wet bulb temperature to the absolute humidity (given the dry bulb temperature). For previous versions of the model, T_w was calculated using an empirical expression ^[124] providing T_w (°C) as a function of T (°C) and % RH. Making these updates, along with using a set of experiments to confirm how well the model prediction and experimental data compared within the constant rate period proved advantageous, and it was found that experimental and predicted % M.C. values compared well, but the model was found to underpredict in one instance and overpredict for the other two experiments within the triplicate work. The difference between the prediction and experimental data was found to be <5% moisture content in each experiment. The highly controlled environment of the DVS compared to the capabilities of the oven has been highlighted as a possible reason for the difficulty in finding repeatability across the triplicate experiments and the % M.C. values obtained. Many of the variables in the oven e.g., pressure, temperature, and % RH show less control when targeting specific conditions when compared to the DVS Intrinsic system. For example, the pressure is set based on reading from a pressure gauge and not digitally set. Small differences here can play a big role on the drying process occurring inside, therefore affecting drying time and % M.C. values of sample.

6.2. Aims of modelling work

The main aims of the work detailed in this chapter include:

- Assessing previously generated experimental DVS data to establish the potential to model the drying rate curve data for both CBZ dihydrate and THEO monohydrate.
- Modelling the drying data from the DVS for CBZ dihydrate and THEO monohydrate.
- Assessing the potential of the modelling approach to provide predictive drying times when drying hydrate APIs in a convective drying environment.
- Scaling up the model to a lab-based convective drying oven.

6.3. Introduction

Within the pharmaceutical space, drying is a commonly utilised operation. However, the theory and models underlying this process have not yet been fully developed. The intent of drying a hydrated form is the removal of additional solvent from a wet product, while preventing de-solvation. Given the necessity of drying across the industry, the overarching aim was to develop a reliable prediction model across a range of scales.

The driving constraint behind the lack of comprehensive drying models to date has been the lack of adequate data from the various apparatuses used. As such a Dynamic Vapor Sorption (DVS) has been utilised to generate highly accurate drying data over the duration of both the drying and dehydration stages of solvent (water) removal. This work was discussed in detail in **CHAPTER 5**. DVS is typically a gravimetric technique that measures both the solvent uptake or removal rate and quantity by varying the vapor concentration surrounding the sample and periodically measuring the sample mass. Experimental datasets resulting from DVS runs

were utilised in the development of a model-enabled platform for the prediction of convective drying parameters.

6.4. Model Details

6.4.1. Experimental Data used for determining X_c and h_c

The DVS data discussed in **CHAPTER 5** for both CBZ dihydrate and THEO monohydrate were used for the calculations of X_c and h_c for both compounds. This work provided a wide range of % RH and temperatures from which to calculate these parameters with no additional experimental work required.

The data sets used were:

- CBZ dihydrate at 30°C, at 10, 20, 40 and 60% RH.
- CBZ dihydrate at 40°C, at 10, 20, 40 and 60% RH.
- THEO monohydrate at 30°C, at 10, 20, 40 and 60% RH.
- THEO monohydrate at 40°C, at 10, 20, 40 and 60% RH.

Please see **Section 6.4.2.1** for details on why 0% RH and 80% RH data sets, which were discussed in **CHAPTER 5**, were not used in the model.

6.4.2. Model Equations

6.4.2.1. Determining X_c and h_c from DVS data

The fundamental drying equation for a wet solid is given by ^[50]:

$$N = -\frac{m_s}{A} \frac{dX}{d\theta} \quad \text{Eq. 1}$$

Where:

N = rate of drying ($kg/m^2/s$)

m_s = mass of dry solid (kg)

A = surface area for evaporation (m^2)

dX = change in moisture content – dry basis
 (% kg water/ kg dry solid)
 $d\theta$ = change in drying rate ($kg\ H_2O\ m^{-2}/s$)

Two drying regions are assumed for this work (see Figure 1.1 below). At moisture contents above a critical value (X_c) the drying rate is assumed to be constant ($= N_c$). Below the critical value, the drying rate is assumed to fall linearly with moisture content. As the APIs used in this study are non-porous crystals, the equilibrium moisture content (X^*) is assumed to be equal to 0.

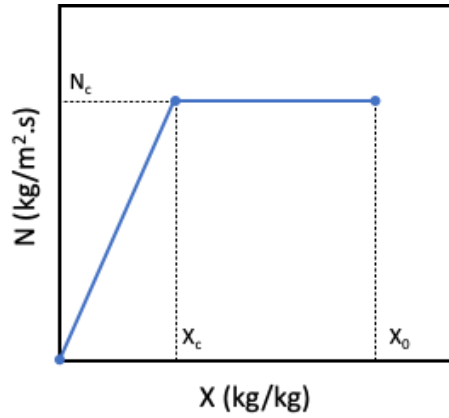


Figure 6.1: Drying rate curve for drying of non-porous crystal.

(i) Constant drying rate period ($X > X_c$ & $N = N_c$).

Integrating equation 1, the time to dry from X_1 to X_2 is given by:

$$\theta = - \int_{X_1}^{X_2} \frac{m_s}{AN_c} dX \quad \text{Eq. 2}$$

which gives:

$$\theta = \frac{m_s(X_1 - X_2)}{AN_c} \quad \text{Eq. 3}$$

(ii) Falling rate period ($X < X_c$)

Assuming linearly falling drying rate, then:

$$N = N_c \frac{(X - X^*)}{(X_c - X^*)} \quad \text{Eq. 4}$$

Integrating equation 1, the time to dry from X_1 to X_2 is given by:

$$\theta = - \int_{X_1}^{X_2} \frac{m_s}{AN} dX \quad \text{Eq. 5}$$

Substituting equation 4 into equation 5 and integrating, gives:

$$\theta = \frac{m_s(X_c - X^*)}{AN_c} \ln \frac{(X_1 - X^*)}{(X_2 - X^*)} \quad \text{Eq. 6}$$

Therefore, **the total drying time** to dry a solid from $X_0 (> X_c)$ to $X_e (< X_c)$ is given by:

$$\theta = \frac{m_s(X_0 - X_c)}{AN_c} + \frac{m_s(X_c - X^*)}{(AN_c)} \ln \frac{(X_c - X^*)}{(X_e - X^*)} \quad \text{Eq. 7}$$

During the constant rate period, when surface evaporation of unbound moisture occurs, the rate of heat transfer to the wet surface is given by:

$$Q = Q_c + Q_R + Q_K \quad \text{Eq. 8}$$

Where:

Q_c = convective heat flux (W)

Q_R = radiative heat flux (W)

Q_K = conductive heat flux (W)

For drying in the DVS, it is assumed that Q_R and Q_K are negligible.

For air at a temperature T_g and a solid surface at a temperature T_s , then

$$\theta = h_c A (T_g - T_s) \quad \text{Eq. 9}$$

Where:

h_c = convective heat transfer coefficient ($W/m^2 \cdot K$)

Heat does two things:

1. It evaporates the moisture at T_s
2. It heats the evaporated moisture from T_s to T_g (the gas temperature).

This change in temperature in the second point above is the so-called “sensible heat load”. It is equal to $C_p(T_g - T_s)$, where C_p is the heat capacity of the gas-vapour mixture. As the value from point 2 is much smaller than the first, it is generally ignored as it really makes no difference to the calculations.

So, ignoring any sensible heat loads:

$$Q_c = \lambda_s A N_c \quad \text{Eq. 10}$$

Where:

λ_s = latent heat of vaporization of water at the solid surface
($W/m^2.K$)

The wet bulb temperature, T_w , is calculated based on the gas temperature and % RH. From this the constant drying rate (N_c) is calculated.

$$N_c = \frac{h_c(T_g - T_s)}{\lambda_s} \quad \text{Eq. 11}$$

Moisture (X) and experimental time (θ_{exp}) data was taken from each DVS data set, for each % RH and temperature. The experimental data was then used to determine the critical moisture content (X_c) and convective heat transfer coefficient (h_c) by comparing the experimental drying times with those predicted by the drying model (equations 3, 6 & 7). **Figure 6.2** shows the systematic approach for calculating these values. For the final step, the error between the experimental measurements and model

predictions is minimised (using the sum of squares) using the Solver function in Excel. Solver is an optimization tool. It finds the optimal solution to a problem by changing multiple variables. In this case, the solution to the problem is reducing the value for the sum of squares to the smallest values, by changing multiple variables, which are the initial 'assumed' values for X_c and h_c .

Residual Sum of Squares (SSQ) of the experimental drying time versus predicted drying time is reduced by varying X_c and h_c using the Solver function in Excel.

$$\sum (t_{exp} - t_{pred})^2 \quad \text{Eq. 12}$$

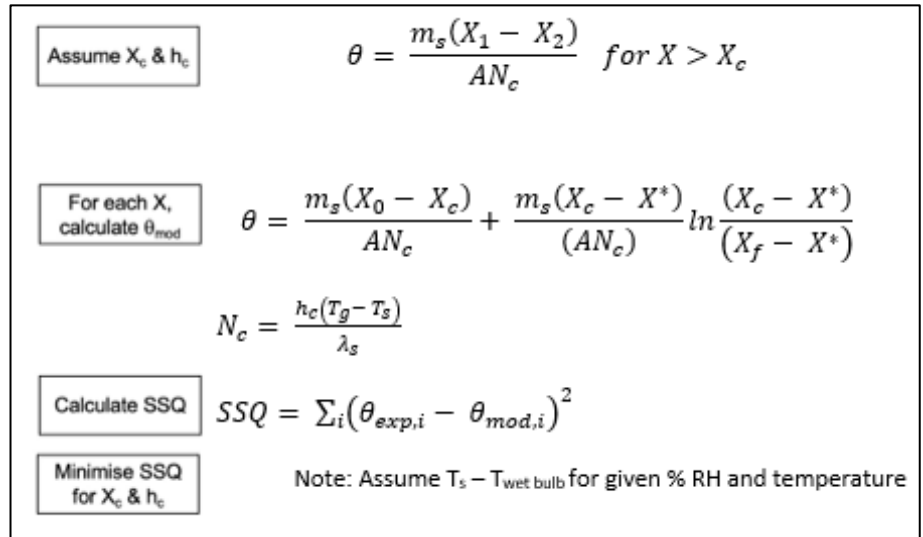


Figure 6.2: Systematic approach to calculating X_c and h_c from DVS data.

The DVS experimental data at 0% RH and 80% RH usually showed long drying times when compared to 10% - 60% RH data sets and this increased the sum of squares. Hence, data from 10-60% RH was used for modelling purposes. Other known inputs of the DVS include the area of the chamber which was 40 mm x 40 mm x 50 mm (W x D x H) and its set gas flow of 200 standard cubic centimetres per minute (sccm), which equals $3.33 \times 10^{-6} \text{ m}^3\text{s}^{-1}$ which equals $0.00188 \text{ kgm}^{-2}\text{s}^{-1}$.

- $\text{m}^3\text{s}^{-1} = (200 \text{ sccm}) (10^{-6}/60) = 3.33 \times 10^{-6} \text{ m}^3/\text{s}$
- $\text{kgm}^{-2}/\text{s} = (\text{density of nitrogen}) (3.33 \times 10^{-6} \text{ m}^3/\text{s})/(\text{area of chamber}) = 0.00188 \text{ kgm}^{-2}/\text{s}$

Model input parameter values used for the case of drying of CBZ dihydrate at 30 °C are provided in **Table 6.1** below.

Table 6.1: Values of calculated parameters used in the model for CBZ dihydrate at 30 °C

DVS Gas Flow			
Parameters		Unit	Value
Density of Nitrogen	ρ_{N_2}	kgm^{-3}	1.127
Area of the Chamber	A_{chamber}	m^2	2.00E-03
Gas flow rate	G	sccm	200
		m^3s^{-1}	3.33E-06
		$\text{kgm}^{-2}\text{s}^{-1}$	1.88E-03
Gas Temperature	T_g	K	303.15
Equilibrium Moisture Content	X^*	-	0
Cross sectional Area of the cake bed	A_{bed}	m^2	3.85E-05
Latent heat of solvent vaporisation	λ	Jkg^{-1}	2430000

The cross-sectional area of the cake bed is given by:

$$\pi \left(\frac{d}{2} \right)^2 = \pi \left(\frac{0.007}{2} \right)^2 = 3.85 \times 10^{-05} \text{ m}^2 \quad \text{Eq. 12}$$

The area of the chamber is given by:

$$\left(\frac{40}{1000} \right) \times \left(\frac{50}{1000} \right) = 0.002 \text{ m}^2 \quad \text{Eq. 14}$$

6.4.2.2. Oven drying time predictions

For the convective drying model, there are several user inputs that are required:

1. Initial Moisture Content (%)
2. Measured Relative Humidity set outside the oven (% RH)
3. Oven pressure (bar)
4. Initial sample mass (g)
5. Gas temperature (°C)
6. Internal (inside the oven) % RH – separately calculated

Parameters numbered 1-5 are obtained when deciding the experiments and during sample preparation. Parameter number 6 is then calculated with some of the user inputs.

The estimated X_c and h_c (**Section 1.4.2.1**) are used to predict the drying time in the oven. The X_c is the same in the oven as in the DVS, whereas the h_c must be scaled up to match the oven conditions. The convective heat transfer coefficient of the oven is a factor of the gas flow rate in the oven. The scale of the gas flowrate in the oven is compared to the DVS.

Model input parameter values used for the case of drying of CBZ dihydrate at 30 °C and 20% RH are provided in **Table 6.2** below.

Table 6.2: Values of calculated parameters used in the model for CBZ dihydrate at 20% RH and 30 °C.

Oven Gas Flow			
Density of Nitrogen	ρ_{N2}	kgm ⁻³	1.127
Dryline velocity		(L/min)	2.4
Wetline velocity		(L/min)	0
Area of the Oven	A_{oven}	m ²	8.41E-02

Gas flow rate	G_{oven}	m^3s^{-1}	4.00E-05
		$kgm^{-2}s^{-1}$	5.36E-04
Gas flow Ratio	x	-	0.534
Cross sectional area of the oven cake bed	$A_{ovencakebed}$	m^2	0.00177
Latent heat of solvent vaporization	λ	Jkg^{-1}	2.43E+06

In the case of the model demonstrated, the following was used:

Gas flowrate of the DVS (G_{DVS}) = 0.00188 kgm^{-2}/s

Gas flowrate of the Oven (G_{Oven}) = 0.000536 kgm^{-2}/s when the dryline velocity = 2.4 L/min and wet line velocity = 0 L/min (when aiming for 20% RH outside the oven).

Gas flow Ratio is given by:

$$\frac{G_{DVS}^{0.5}}{G_{Oven}^{0.5}} \quad \text{Eq. 15}$$

Heat transfer coefficient (oven) is given by:

$$h_{c,Oven} = h_{c,DVS} \cdot \text{Gas flow Ratio} \quad \text{Eq. 16}$$

Theoretical mass of moisture is given by:

$$CBZ_{moist} = \frac{M_0}{100} \cdot X_0 \quad \text{Eq. 17}$$

Where:

M_0 = Initial sample weight (g)

X_0 = Initial moisture content (%)

Theoretical mass of dehydrated form is given by:

$$CBZ_{dehy} = M_0 - CBZ_{moist} \quad \text{Eq. 18}$$

Theoretical mass of hydrate/solvate is given by:

$$CBZ_{moist} = CBZ_{dehy} \cdot \frac{Mw_{compound} + Mw_{solvent}}{Mw_{compound}} \quad \text{Eq. 19}$$

To determine the oven drying time, the wet bulb temperature inside the oven (T_w) must be calculated. For this, the relative humidity in the oven must be recalculated, as what was targeted on the outside is affected by the temperature and oven pressure on the inside. For the wet bulb temperature, the following calculations were carried out:

The saturated vapour pressure outside the oven was calculated at the gas temperature using the Antoine equation, which is given by:

$$\log_{10}P = A - \frac{B}{C+T} \quad \text{Eq. 20}$$

Where :

T = Temperature ($^{\circ}\text{C}$)

P = Pressure (mmHg)

A, B and C are constants for water, see **Table 6.3** below.

Table 6.3: Table of Constants for the Antoine Equation. ^[125]

Formula	Name	A	B	C	$T_{\min}$ ($^{\circ}\text{C}$)	$T_{\max}$ ($^{\circ}\text{C}$)
Ar	Argon	6.84064	340.271	271.801	-189.37	-122.29
CO	Carbon monoxide	6.72527	295.228	268.243	-205.00	-140.23
CO ₂	Carbon dioxide	7.58828	861.82	271.883	-56.57	31.04
H ₂	Hydrogen	6.14858	80.948	277.532	-259.2	-239.97
H ₂ O	Water	8.05573	1723.64	233.076	0.01	373.98
H ₂ S	Hydrogen sulfide	7.11958	802.227	249.61	-85.47	100.38
N ₂	Nitrogen	6.72531	285.573	270.087	-210.00	-147.05
N ₂ O	Nitrous oxide	6.81488	550.604	228.438	-90.85	36.42
NO	Nitric oxide	7.70299	473.03	249.866	-163.65	-93.00
O ₂	Oxygen	6.83706	339.209	268.702	-218.8	-118.57
SO ₂	Sulfur dioxide	7.33311	1013.46	237.647	-75.48	157.6

At gas temperature (T_g), saturated vapour pressure (P_{vap}) is calculated using the Antoine equations. At atmospheric pressure (outside the oven) ($P_{out} = 101325$ Pa), the saturated humidity ($Y'_{sat\_outside}$) can be calculated as:

$$Y'_{sat\_outside} = \frac{P_{vap}}{P_{out}-P_{vap}} \left(\frac{Mw_{water}}{Mw_{N_2}} \right) \quad \text{Eq. 21}$$

From this, the absolute humidity ($Y'_{outside}$) is calculated as follows:

$$Y'_{outside} = (Y'_{sat\_outside}) * \frac{RH\%}{100} \quad \text{Eq. 22}$$

Note the absolute humidity outside = absolute humidity in the oven, Y'_{oven}

The wet bulb temperature (T_w) can be calculated using:

$$T_w = T_G - \frac{\lambda}{\frac{h_G}{K_y}} (Y'_w - Y') \quad \text{Eq. 23}$$

For air- water systems, the ratio $\frac{h_G}{k_y}$ can be taken to be 950 N.m/kg.K, and the wet bulb temperature can be calculated using:

$$T_w = T_G - \frac{\lambda}{950} (Y'_w - Y') \quad \text{Eq. 24}$$

Here, Y'_w is a function of T_w .

$$\frac{h_G}{k_y C_S} = \left(\frac{S_C}{P_r} \right)^{0.567} = Le^{0.567} \quad \text{Eq. 25}$$

Where:

$$S_C = \frac{\mu}{e^{D_{AB}}}$$

$$P_r = \frac{\mu}{e^k} = 0.7$$

Using trial and error, T_w is determined.

At first, a T_w value was estimated. Using this T_w , saturated vapour pressure at T_w is calculated (Antoine equation, Eq. 20). The saturated humidity at T_w is determined as follows:

$$Y'_{sat_{T_w}} = \frac{P_{vap_{T_w}}}{P_{oven}-P_{vap_{T_w}}} \left(\frac{Mw_{water}}{Mw_{N_2}} \right) \quad \text{Eq. 26}$$

The absolute humidity at T_w was calculated as follows:

$$Y'_{Tw} = Y'_{sat_{Tw}} * \frac{RH\%}{100} \quad \text{Eq. 27}$$

Eq. 24 was rearranged as below, and using Goalseek function in Excel, T_w was calculated=

$$0 = T_G - T_w - \frac{\lambda}{950} (Y'_w - Y') \quad \text{Eq. 28}$$

Goalseek is the opposite of a function and instead of finding the end result, the user gives the result, and it determines the input. Using Goalseek, the right-hand side of the equation is set to zero by changing the T_w . Hence, T_w is determined.

The constant rate period used for the prediction of oven drying times was calculated using:

$$N_c = h_c (T_g - T_w) \left(\frac{A_{bed}}{\lambda} \right) \quad \text{Eq. 29}$$

From here, the drying time (prediction) is calculated combining the below two 'If' statements, using Excel:

$$(i) \quad \text{If } X > X_c, \theta = \frac{m_s(X_0 - X)}{AN_c} \quad \text{Eq. 30}$$

$$(ii) \quad \text{If } X < X_c, \theta = \frac{m_s}{AN_c} \left[(X_0 - X_c) + (X_c - X^*) \ln \left(\frac{X_c - X^*}{X - X^*} \right) \right] \quad \text{Eq. 31}$$

6.5. Results

Section 6.5.1, 6.5.2 and 6.5.3 detail experiments using earlier versions of the model, showing the development of the calculations used and the issues encountered, while **Section 6.5.4** details the validation experiments for the final version of the model developed and which is thoroughly discussed in **Section 6.4**.

6.5.1. Vapour pressure comparison experiments using THEO monohydrate – early model predictions

Examining the differences between the DVS and the oven raised the question of the effects of the oven operating pressure on the % RH being experienced inside the oven. This in turn led to examining the vapour pressure values based on the set conditions. A vacuum oven was used in the work described in this chapter (**Section 2.10**), and with that, pressure and humidity values within the oven are reduced, however the effects of the difference between % RH set outside and the % RH experienced inside the oven had not previously been considered. After noting that this difference may be significant, investigations into vapour pressure values were needed. In this Section, head-to-head experiments were carried out to test whether two experiments with different system conditions set on the outside but with the same vapour pressure value inside the oven, gave similar results, in terms of % moisture content after drying. Results from this would determine whether % RH alone (which was incorporated into the model used for predicting the drying times of these experiments) or using the % RH value to determine vapour pressure values would be incorporated into the next version of the model.

Another point to note here, as this was an earlier version of the model, the wet bulb temperature (T_w) was also calculated differently to what is detailed in **Section 6.4.2.1**. for this earlier version of the model. This was calculated using an empirical expression, as follows ^[124]:

$$T_w = ((T_g) * \text{ATAN}(0.151977 * (\%RH + 8.313659)^{0.5}) + \text{ATAN}((T_g) + \%RH) - \text{ATAN}(\%RH - 1.676331) + 0.00391838 * (\%RH^{(3/2)})) * \text{ATAN}(0.023101 * \%RH) - 4.686035) \quad \text{Eq. 32}$$

Where:

T_g = gas temperature of the experiment

% RH = % RH set outside

The arctangent (ATAN) function uses the argument values as though they are in radians. Values calculated from Eq. 29 show T_w as an explicit function of T and % RH. The limiting factor of this equation and why it is not used in the final version of the model (detailed later) is because it is only valid at a pressure of 101.325 kPa. As development work on the model continued, the need to incorporate the oven pressure became a key factor for predicting drying times.

6.5.1.1. Drying at 20°C, 40% RH and 1.00 bar vs. 20°C, 80% RH and 0.50 bar

Table 6.4 shows the comparison of conditions for the first set of experiments to be discussed. While the system conditions differ, the vapour pressure being experienced by the sample is the same based on the fact that the moisture content (mole fraction) of the air in the feed is the same as in the oven:

$$\text{Mole fraction} = \frac{p_f}{P_F} = \frac{p_o}{P_O} \quad \text{Eq. 33}$$

Where:

p_f = partial pressure in feed

P_F = pressure of feed

p_o = partial pressure in oven

P_O = pressure in oven

$$\text{From this, } p_o = p_f \left(\frac{P_O}{P_F} \right)$$

Since P_F is constant, then if p_o increases, P_O should be decreased.

This calculation was done separately to the model so vapour pressures were not incorporated into the model in this instance.

The aim of these experiments was to compare the drying of two samples that were experiencing the same levels of vapour pressure inside the oven, despite their outside conditions being different. One set of conditions (condition A in the following tables) was used to predict the drying time via the model. For the second experiment and second set of conditions (condition B in the following tables) the % RH outside the oven and pressure inside the oven were adjusted (such that the vapour pressure inside the oven should be the same as under the first set of conditions) and the sample was dried for the same amount of time as for the first experiment.

A batch of wet THEO monohydrate was dried during these experiments with preparation details found in Chapter X Section X. Each set of system conditions were allowed to equilibrate for 1 hr prior to adding a sample into the oven. Both experiments were carried out the same day meaning the pre-drying % total Moisture Content was the same in both experiments. This value was obtained using the Moisture Analyzer, see **Section 2.5.2**.

Table 6.4: Comparison of conditions and inputs for the first set of head-to-head experiments for oven drying of THEO monohydrate.

	Condition 1A	Condition 1B
	20°C, 40% RH and 1.00 bar	20°C, 80% RH and 0.50 bar
T_g (°C)	20.00	20.00
Measured outside RH (%)	40.00	80.00
Oven Pressure (bar)	1.00	0.50
Pre-drying MC (%)	43.60	43.60
Initial sample weight (g)	1.26267	1.26217
X*	0.00	0.00

Note: * As the APIs used in this study are non-porous crystals, the equilibrium moisture content (X^*) is assumed to be equal to 0.

In parallel to this practical work, this version of the model was successfully converted into a user friendly WebApp by a colleague as part of a separate project. Prior to the oven drying prediction details shown in **Figure 6.3** being generated, the experimental tab of the WebApp was used to upload DVS data for the relevant compound, which was THEO monohydrate for these experiments. This DVS data includes all the raw data files from the drying study experiments discussed in **CHAPTER 5**. Once the DVS data was uploaded, it was used to calculate the X_c and h_c , based on the equations (Eq.1-12) and the systematic approach detailed in **Figure 6.2**.

Experimental conditions used as model inputs, e.g. temperature, %RH, sample weight, initial % moisture content are included in **Figure 6.3** under 'Input conditions for oven drying'. By clicking 'Update prediction', the 'Final Time', 'Final Moisture Content' and 'Final Mass' and the drying curve become populated. Using the conditions of the first experiment (Table 1.4, Condition 1A), 20 °C, 40% RH and 1.00 bar, a predicted drying time (to arrive at the dry hydrate) of 201 min was calculated.

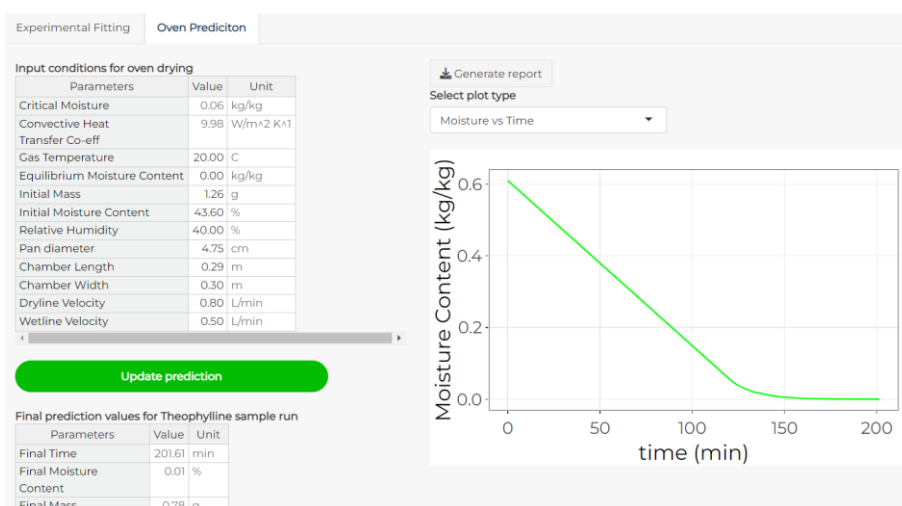


Figure 6.3: WebApp (version 1, in-house development) of the model, predicting the drying time of the experiment at 20°C, 40% RH and 1.00 bar for THEO monohydrate.

Table 6.5 shows a summary for the experiment at 20°C, 40% RH and 1.0 bar, with the post drying, % Moisture Content value. After drying a sample of THEO monohydrate for 201 min, the sample contained 17.26% Moisture Content which is significantly higher than the desired value of 9.08% which correlates to the monohydrate alone, with no excess, loosely bound moisture.

Table 6.5: Overview of results for the oven drying experiment at 20°C, 40% RH and 1.0 bar – THEO monohydrate.

20°C, 40% RH and 1.00 bar - THEO monohydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	17.26
<i>Desired M.C. (%)</i>	9.08

This version of the model underpredicted the drying at 20°C, 40% RH and 1.00 bar. However, as the main aim of this work was to compare the drying with another experiment, where the vapour pressure value inside the oven was the same, the second experiment at 20°C, 80% RH, 0.5 bar was conducted.

The system conditions and sample details for the experiment at 20°C, 80% RH and 0.50 bar can be seen in **Table 6.4** (Conditions 1B). Based on Eq. 30, as the % RH was doubled, in order to keep the vapour pressure inside the oven the same, the pressure of the oven needs to be halved. The predicted drying time of the previous experiment (20°C, 40% RH and 1.00 bar) detailed in **Figure 6.3**, of 201 min, was used as the drying time for this second sample. This allowed for a comparison of the two samples which were experiencing equal vapour pressure values but different system conditions.

Results for the experiment at 20°C, 80% RH and 0.50 bar, after drying for 201 min are shown in **Table 6.6** and show an end point sample with a % Moisture Content of 9.68%. This is closer to the desired value of 9.08% for the monohydrate.

Table 6.6: Overview of results for the oven drying experiment at 20°C, 80% RH and 0.5 bar – THEO monohydrate.

20°C, 80% RH and 0.50 bar – THEO monohydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	9.68
<i>Desired M.C. (%)</i>	9.08

Early developmental work showed that the model tended to underpredict drying times when experiments were undertaken at atmospheric pressure and the experiment at 20°C, 80% RH and 1.00 bar appears to also show the same issue. In hindsight, doing an experiment at this pressure was incorrect as it is not representative of how the oven would be used. In contrast, the experiment at 20°C, 80% RH and 0.50 bar resulted in a sample retrieved which had a % Moisture Content close to that of the dry monohydrate. While a promising result with regards the predicted drying time, there were still questions unanswered regarding the use of the vapour

pressure values (as vacuum wasn't used in the experiment at 20°C, 80% RH and 1.00 bar) and whether the higher % RH of the latter experiment, even at 0.50 bar, was providing an environment which allowed for hydrate stability and whether the sample was removed during that period.

6.5.1.2. Drying at 20°C, 20% RH and 0.50 bar vs. 20°C, 40% RH and 0.25 bar

The same objective detailed in **Section 1.5.1.1** was set for the following two experiments, i.e., to determine whether different system conditions which give equal vapour pressure values inside the oven, based on Eq. 30, experience the same level of drying when dried for the same amount of time based on the model. However, for these experiments, both were carried out under a level of vacuum.

Table 6.7 shows the comparison of conditions for both experiments to be discussed. Again, wet THEO monohydrate was dried during these experiments. The system was allowed to equilibrate for 1 hr prior to adding a sample. Sample preparation for these experiments is detailed in Section 2.10.1.3.

Table 6.7: Comparison of conditions and inputs for the second set of head-to-head experiments for oven drying of THEO monohydrate.

	Condition 2A	Condition 2B
	20°C, 20% RH and 0.50 bar	20°C, 40% RH and 0.25 bar
T_g (°C)	20.00	20.00
Measured outside RH (%)	20.00	40.00
Oven Pressure (bar)	0.50	0.25
Pre-drying MC (%)	38.46	40.33

Initial sample weight (g)	1.04668	1.42500
X^*	0.00	0.00

Note: * As the APIs used in this study are non-porous crystals, the equilibrium moisture content (X^*) is assumed to be equal to 0.

The same WebApp was used to predict the drying time for the experiment at 20°C, 20% RH and 0.50 bar (as shown in **Figure 6.4**), and gave a drying time of 59 min.

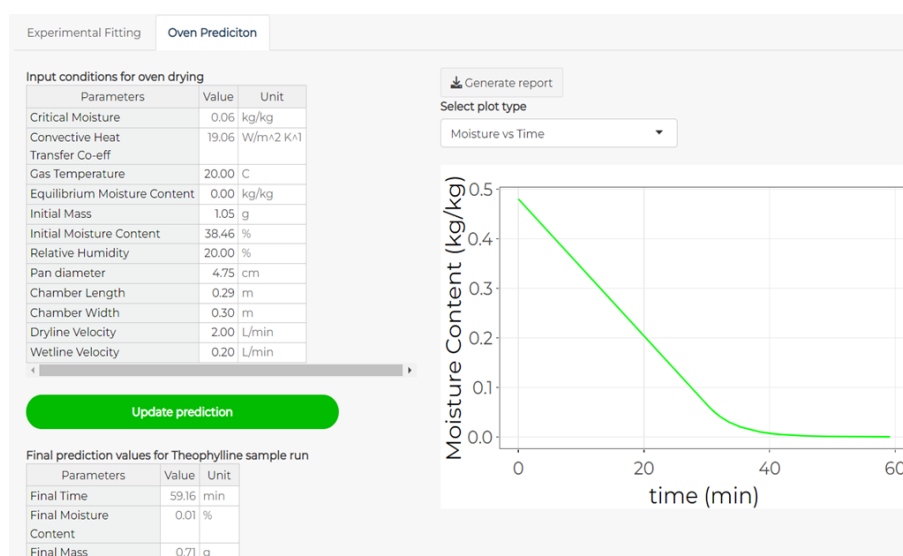


Figure 6.4: WebApp (version 1, in-house development) of the model, predicting the drying time of the experiment at 20°C, 20% RH and 0.50 bar for THEO monohydrate.

Table 6.8 shows a summary result for the experiment 20°C, 20% RH and 0.50 bar, with the post drying, % Moisture Content value. After drying for 59 min, the sample contained 13.33% total Moisture Content which is higher than the desired value of 9.08% which correlates to the dry monohydrate.

Table 6.8: Overview of inputs and results of the oven drying experiment at 20°C, 20% RH and 0.50 bar – THEO monohydrate.

20°C, 20% RH and 0.50 bar – THEO monohydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	13.33
<i>Desired M.C. (%)</i>	9.08

This model was still underpredicting drying time. However, results could still be used for comparison with the experiment at 20°C, 40% RH and 0.25 bar.

The system conditions and sample details for the experiment can be seen in **Table 6.7** (Condition 2B). Based on Eq. 30, as the % RH was doubled from 20% RH to 40% RH in this second experiment, in order to keep the vapour pressure inside the oven the same, the pressure of the oven needs to be halved from 0.50 bar to 0.25 bar. For this experiment, the same predicted drying time detailed in **Figure 6.4** (59 min) was used.

Results for the experiment at 20°C, 40% RH and 0.25 bar after drying for 59 min are shown in

Table 6.9 and a significant underprediction of drying time is apparent. The % Moisture Content contains ~9% excess, loosely bound moisture which was not removed within the 59 min.

Table 6.9: Overview of inputs and results of the oven drying experiment at 20°C, 40% RH and 0.25 bar – THEO monohydrate.

20°C, 40% RH and 0.25 bar – THEO monohydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	18.26
<i>Desired M.C. (%)</i>	9.08

Underprediction of the drying time once again proved to be an issue. However, ultimately both experiments were dried to different extents even though the same environment (in terms of vapour pressure) was present. At this point, the calculations within the model were examined more closely and it was found that the oven pressure values were not actually being incorporated into any calculation. This meant that the effects of the vacuum were not being accounted for when predicting the drying time using this version of the model.

After fully reviewing the calculations within the model, the unsuccessful results of **Section 6.5.1.1** and **6.5.1.2** are likely to be due to the fact that the pressure reading of oven is not included. This early oversight was likely because operating pressures didn't play a role in DVS experiments. From here, it raised the question of how % RH, vapour pressure and the oven pressure values should be incorporated and calculated within the model.

6.5.2. Adjusted model with calculation for % RH inside the oven

6.5.2.1. CBZ Dihydrate – 30°, 20% RH and 0.25 bar

Underprediction of drying times from the model was consistently seen across previous experiments and while conditions (temperature, % RH of in-going gas, pressure) going into the oven were being tracked and monitored, to what level these same conditions were being experienced inside the oven was brought into question, especially in relation to the level of % RH.

Coupling together the idea of using vapour pressure values rather than the % RH value set on the outside, the Antoine equation provided a new insight into the environment being experienced inside the oven depending on the conditions set on the outside. The Antoine equation is a correlation which expresses vapour pressure as a function of temperature.

By using the table of constants, **Table 6.3**, the values for water are used in Eq. 20-22. They allow for the % RH inside the oven to be calculated. This calculation provided an update to the model discussed in **Sections 6.5.1.1** and **6.5.1.2**. With this new % RH value, for inside the oven, the T_w value was calculated using an empirical expression ^[124] providing T_w (°C) as a function of T (°C) and % RH. This value then feeds into Eq. 29-31 to determine the predicted drying time. Due to this update, the earlier designed WebApp became obsolete, but it was intended that a new version would be developed once a final version of the model had been validated. For the remainder of the work discussed in this chapter, updated versions of the original model, were built, and used in Excel, with the predicted drying times being taken from there.

Figure 6.5 shows a screenshot of the working model, whereby the 'RH inside the oven (%)' is calculated as discussed above. The

experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.10** below:

Table 6.10: Conditions and inputs for the oven drying experiment at 30°C, 20% RH and 0.25 bar – CBZ dihydrate

	30°C, 20% RH and 0.25 bar
T_g (°C)	30.00
Measured outside RH (%)	20.00
Oven Pressure (bar)	0.25
Pre-drying MC (%)	61.97
Initial sample weight (g)	1.09882
X*	0.00

With the Antoine equation being used, the predicted drying time for this sample was 86 min (**Figure 1.7**). After the experiment, the Moisture Analyzer was used to get a % Moisture Content value immediately after being removed from the oven (after 86 min). Due to small sample size and to ensure enough sample was available for additional analyses, this analysis was carried out singularly.

T _g (C)	30	User input	Total Drying time (min)			
Measured outside RH (%)	20		Predicted		85.71	
Oven Pressure (bar)	0.25					
Initial weight (g)	1.09882		Prediction			
Pre drying MC (%)	61.97		X	Time (s)	Time (min)	Time (h)
X* (-)	0.00		1.28	0	0	0.00
RH inside oven (%)	2.352740031		0.78	1200.44	20.0	0.33
T wet bulb (C)	10.08		0.28	2400.88	40.0	0.67
Initial wet solid (g)	1.09882		0.18	2640.97	44.0	0.73
hydrate dry wt (g)	0.481608096		0.08	2904.03	48.4	0.81
Gas Temperature (K)	303.15	0.03	3187	53.1	0.89	
Wet Bulb Temperature (K)	283.23	0.02	3300.56	55.0	0.92	
Initial wet solid (kg)	1.10E-03	0.01	3486.29	58.1	0.97	
hydrate dry wt (kg)	4.82E-04	0.001565	4082.52	68.0	1.13	
Initial moisture content (-)	1.28	0.000565	4386.32	73.1	1.22	
Critical Moisture Content (X _c) (-)	0.124	From DVS	0.000065	5032.37	83.9	1.40
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	13.809	From DVS	0.000055	5082.47	84.7	1.41
Constant Drying rate (kgs ⁻¹)	2.01E-07		0.000045	5142.72	85.7	1.43
MW CBZ (g/mol)	236.269					
MW CBZ-DH (g/mol)	272.3					
Theoretical wt of all moisture (g)	0.680938754					
Theoretical endpoint wt (Anhy/dehy) (g)	0.417881246					
Theoretical wt of dry dihydrate (g)	0.481608096					

Figure 6.5: Screenshot of model, via Excel, predicting the drying time of the experiment at 30°, 20% RH and 0.25 bar for CBZ dihydrate with updated calculation for the % RH inside the oven being highlighted.

Table 6.11 shows that the end point sample had a % Moisture Content of 13.16%, which is close to the desired 13.22% for a dry CBZ dihydrate. This was a promising result. This end point sample also had an XRPD trace which overlaid very well with the dihydrate (**Figure 6.6**). The trace of the end point sample was more resolved than that of the dihydrate trace, but the characteristic peaks are present in both.

The calculations used in this updated model gave an internal % RH value of 2.35% which is a very low level of humidity and eliminates the possible argument of the sample being removed within a period of hydrate stability. DVS results previously showed very fast drying times and no stability below 20% RH, suggesting the predicted drying time was correct in determining when all loosely bound moisture had been removed.

Table 6.11: Overview of inputs and results of the oven drying experiment at 30°, 20% RH and 0.25 bar – CBZ dihydrate.

30°, 20% RH and 0.25 bar – CBZ dihydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	13.16
<i>Desired M.C. (%)</i>	13.22

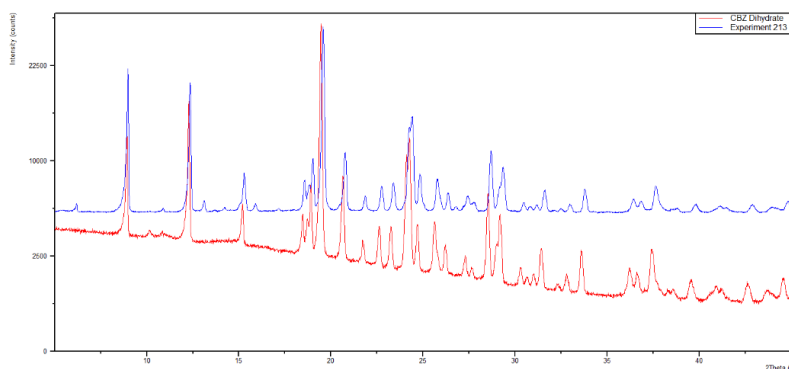


Figure 6.6: XRPD overlay of CBZ dihydrate (red) and the end point sample (blue) of the oven drying experiment at 30°, 20% RH and 0.25 bar.

6.5.2.2. THEO Monohydrate – 20°C, 40% RH and 0.25 bar

A similar experiment was setup with a sample of THEO monohydrate, using the same calculations as discussed in **Section 6.5.2.1**.

Figure 6.7 shows a screenshot of the working model, whereby the ‘RH inside the oven (%)’ is calculated as discussed above. The experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.12** below:

Table 6.12: Conditions and inputs for oven drying experiment at 20°C, 40% RH and 0.25 bar -THEO monohydrate.

	20°C, 40% RH and 0.25 bar
T_g (°C)	20.00

Measured outside RH (%)	40.00
Oven Pressure (bar)	0.25
Pre-drying MC (%)	37.08
Initial sample weight (g)	0.6209
X*	0.00

With the adjusted value for the %RH being experienced inside the oven being incorporated, the predicted drying time for this sample was 43 min. After the experiment, % Moisture Content value was obtained immediately after being removed from the oven (after 43 min). Due to small sample size, this analysis was carried out singularly.

T _g (C)	20	User input	Total Drying time (min)			
Measured outside RH (%)	40		Predicted	43.16		
Oven Pressure (bar)	0.25					
Initial weight (g)	0.6209		Prediction			
Pre drying MC (%)	37.08		X	Time (s)	Time (min)	Time (h)
X* (-)	0.00		0.44	0	0	0.00
RH inside oven (%)	10.48749168		0.34	259.69	4.33	0.07
T wet bulb (C)	7.08		0.24	519.38	8.66	0.14
Initial wet solid (g)	0.6209		0.14	779.07	12.98	0.22
hydrate dry wt (g)	0.429736441		0.04	1564.13	26.07	0.43
Gas Temperature (K)	293.15		0.03	1647.87	27.46	0.46
Wet Bulb Temperature (K)	280.23		0.02	1751.30	29.19	0.49
Initial wet solid (kg)	6.21E-04		0.0148	1895.22	31.59	0.53
hydrate dry wt (kg)	4.30E-04		0.0098	2002.28	33.37	0.56
Initial moisture content (-)	0.44		0.0048	2177.74	36.30	0.60
Critical Moisture Content (X _c) (-)	0.088	From DVS	0.0038	2233.34	37.22	0.62
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	16.305	From DVS	0.0028	2305.03	38.42	0.64
Constant Drying rate (kgs ⁻¹)	1.65E-07		0.0018	2407.04	40.12	0.67
MW THEO (g/mol)	180.164		0.0008	2589.31	43.16	0.72
MW THEO-MH (g/mol)	198.18		0.0003	2798.06	46.63	0.78
Theoretical wt of all moisture (g)	0.23022972		0.0002	2878.33	47.97	0.80
Theoretical endpoint wt (Anhy/dehy) (g)	0.39067028		0.0001	3002.63	50.04	0.83
Theoretical wt of dry monohydrate (g)	0.429736441		0.00004	3293.57	54.89	0.91

Figure 6.7: Screenshot of model, via Excel, predicting the drying time of the experiment at 20°C, 40% RH and 0.25 bar for THEO monohydrate, with updated calculation for the % RH inside the oven being highlighted.

Table 6.13 shows that the end point sample had a % Moisture Content of 9.70%, which is close to the desired 9.08% for dry THEO monohydrate. This was a promising result and coupled with the previous section, these were the closest results achieved to this point. This end point sample also had an XRPD trace which overlaid very well with the monohydrate, **Figure 6.8**. The calculated internal % RH value of 10.49% is a low level of humidity and DVS results for this compound previously detailed fast drying times, with the dry monohydrate having minimal stability below 20% RH. While this work is on a bigger scale than the DVS work, had the sample been over or under dried and the model been incorrect, the low % RH used makes the margin for error and failure narrow, suggesting the model worked correctly in predicting the drying time.

Table 6.13: Overview of inputs and results of the oven drying experiment at 20°C, 40% RH and 0.25 bar – THEO monohydrate.

20°C, 40% RH and 0.25 bar – THEO monohydrate	
<i>Post drying</i>	
<i>End point M.C. (%)</i>	9.70
<i>Desired M.C. (%)</i>	9.08

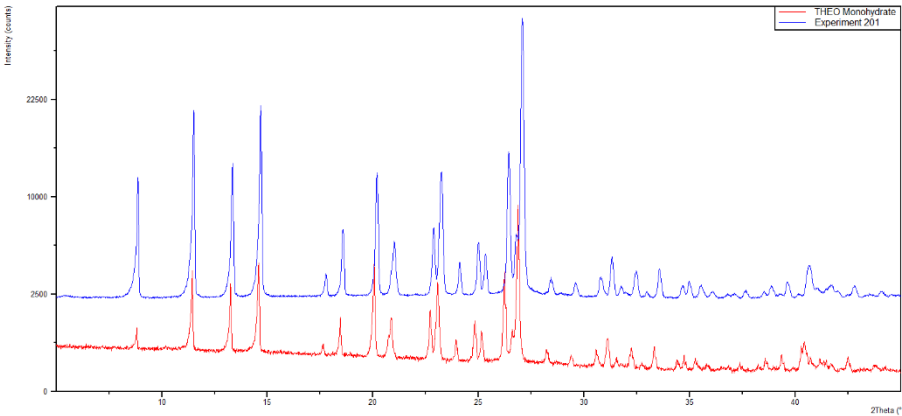


Figure 6.8: XRPD overlay of THEO monohydrate (red) and the end point sample (blue) of the oven drying experiment at 20°C, 40% RH and 0.25 bar.

This update to the model resulted in successful end point % Moisture Content values and the issue of underpredicting was not seen.

6.5.3. Model Testing: Using the minimum pressure for the heating element of the oven to work

6.5.3.1. CBZ Dihydrate

6.5.3.1.1. Drying at 30°C, 20% RH and 0.20 bar

The oven being used for this work was a Binder VDL 23 which required a minimum level of pressure (≤ 0.2 bar) for the heating element to work. Initial validation work of the model was carried out at lower temperatures. These lower temperature experiments were considered familiarisation runs, allowing enough time to understand the system setup and to prepare for additional analysis required once material was removed from the oven post drying. After this, higher temperatures needed to be investigated and with that, a lower pressure reading.

This next experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.14** below:

Table 6.14: Conditions and inputs for the oven drying experiment at 30°C, 20% RH and 0.20 bar – CBZ dihydrate

	30°C, 20% RH and 0.20 bar
T_g (°C)	30.00
Measured outside RH (%)	20.00
Oven Pressure (bar)	0.20

Pre-drying MC (%)	62.50
Initial sample weight (g)	1.01941
X*	0.00

T _ε (C)	30	User input	Total Drying time (min)			
Measured outside RH (%)	20		Predicted		87.19	
Oven Pressure (bar)	0.2		Prediction			
Initial weight (g)	1.01941		X	Time (s)	Time (min)	Time (h)
Pre drying MC (%)	62.5		1.31	0	0	0.00
X* (-)	0.00		0.81	1112.54	18.5	0.31
RH inside oven (%)	1.789191206		0.31	2225.08	37.1	0.62
T wet bulb (C)	10.24		0.21	2447.59	40.8	0.68
Initial wet solid (g)	1.01941		0.11	2671.11	44.5	0.74
hydrate dry wt (g)	0.440576223		0.06	2830.95	47.2	0.79
Gas Temperature (K)	303.15		0.01	3253.74	54.2	0.90
Wet Bulb Temperature (K)	283.39		0.003811	3609.44	60.2	1.00
Initial wet solid (kg)	1.02E-03		0.002811	3693.52	61.6	1.03
hydrate dry wt (kg)	4.41E-04		0.001811	3814.98	63.6	1.06
Initial moisture content (-)	1.31		0.000811	4036.96	67.3	1.12
Critical Moisture Content (X _c) (-)	0.124	From DVS	0.000311	4301.87	71.7	1.19
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	13.741	From DVS	0.000211	4409.14	73.5	1.22
Constant Drying rate (kgs ⁻¹)	1.98E-07		0.000111	4586.88	76.4	1.27
MW CBZ (g/mol)	236.269		0.000011	5231.23	87.2	1.45
MW CBZ-DH (g/mol)	272.3		0.000001	5967.46	99.5	1.66
Theoretical wt of all moisture (g)	0.63713125					
Theoretical endpoint wt (Anhy/dehy) (g)	0.38227875					
Theoretical wt of dry dihydrate (g)	0.440576223					

Figure 6.9: Input section of model predicting the drying time for the oven drying experiment for CBZ dihydrate at 30°C, 20% RH and 0.20 bar.

Using the same updated calculations as in **Section 6.5.2**, the predicted drying time for this sample was 87 min. After the experiment, % Moisture Content value was obtained immediately after being removed from the oven. This analysis was completed in duplicate, with results shown in **Table 6.15**. The end point sample had an average % Moisture Content value of 12.77±0.16% which is slightly lower than the expected value of 13.22% for CBZ dihydrate. XRPD analysis of the sample, when overlaid with the dihydrate form is shown in **Figure 6.10**. The dihydrate trace has some noise along the baseline and the intensity of the end point sample for the experiment at 30°C, 20% RH and 0.20 bar, is reduced in comparison, but the characteristic peaks of the dihydrate are still present.

Table 6.15: Overview of inputs and results for the oven drying experiment at 30°C, 20% RH and 0.20 bar – CBZ dihydrate.

30°C, 20% RH and 0.20 bar – CBZ dihydrate	
<i>Post drying</i>	
<i>Av. End point M.C. (%)</i>	12.77±0.16
<i>Desired M.C. (%)</i>	13.22

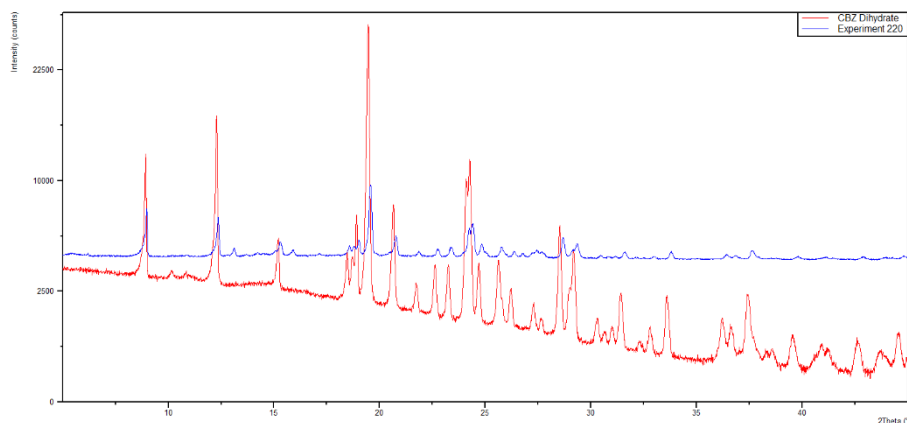


Figure 6.10: XRPD overlay of CBZ dihydrate (red) and the end point sample (blue) of the oven drying experiment at 30°C, 20% RH and 0.20 bar.

The initial % Moisture Content of this sample was high, and this resulted in a long drying time. Adjusting the pressure to 0.20 bar allowed for the temperature to be maintained at 30 °C throughout. Ensuring temperature control, by using this minimum pressure value will be important for higher temperature experiments. Without the temperature control, long drying time experiments would see a slow decrease in the oven temperature over time. The model appears to have slightly overpredicted the required drying time at these conditions.

6.5.3.1.2. Drying at 40°C, 20% RH and 0.20 bar

Another experiment with CBZ dihydrate was set up, at a higher temperature of 40 °C, to test both the model and whether the

pressure value set and used in the model correctly maintains the temperature for the entire experiment.

The experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.16** below:

Table 6.16: Conditions and inputs for the oven drying experiment at 40°C, 20% RH and 0.20 bar - CBZ dihydrate.

	40°C, 20% RH and 0.20 bar
T_g (°C)	40.00
Measured outside RH (%)	20.00
Oven Pressure (bar)	0.20
Pre-drying MC (%)	62.50
Initial sample weight (g)	1.01031
X*	0.00

T _g (C)	40	User input	Total Drying time (min)			
Measured outside RH (%)	20		Predicted	42.62		
Oven Pressure (bar)	0.2					
Initial weight (g)	1.01031		Prediction			
Pre drying MC (%)	62.5		X	Time (s)	Time (min)	Time (h)
X* (-)	0.00		1.31	0	0	0.00
RH inside oven (%)	0.82826747		0.81	617.157	10.3	0.17
T wet bulb (C)	14.79		0.31	1234.31	20.6	0.34
Initial wet solid (g)	1.01031		0.21	1357.75	22.6	0.38
hydrate dry wt (g)	0.436643317		0.11	1481.18	24.7	0.41
Gas Temperature (K)	313.15		0.06	1550.51	25.8	0.43
Wet Bulb Temperature (K)	287.94		0.01	1727.84	28.8	0.48
Initial wet solid (kg)	1.01E-03		0.003811	1877.03	31.3	0.52
hydrate dry wt (kg)	4.37E-04		0.002811	1912.3	31.9	0.53
Initial moisture content (-)	1.31		0.001811	1963.24	32.7	0.55
Critical Moisture Content (X _c) (-)	0.094	From DVS	0.000811	2056.34	34.3	0.57
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	19.055	From DVS	0.000311	2167.46	36.1	0.60
Constant Drying rate (kgs ⁻¹)	3.54E-07		0.000211	2212.45	36.9	0.61
MW CBZ (g/mol)	236.269		0.000111	2287	38.1	0.64
MW CBZ-DH (g/mol)	272.3		0.000011	2557.26	42.6	0.71
Theoretical wt of all moisture (g)	0.63144375					
Theoretical endpoint wt (Anhy/dehy) (g)	0.37886625					
Theoretical wt of dry dihydrate (g)	0.436643317					

Figure 6.11: Input section of model predicting the drying time for the oven drying experiment for CBZ dihydrate at 40°C, 20% RH and 0.20 bar.

The predicted drying time for this sample, at these conditions was 43 min. % Moisture Content analysis was completed in duplicate, as shown in **Table 6.17**. The end point sample had an average % Moisture Content value of $17.03 \pm 0.36\%$ which is more than the expected value of 13.22% for CBZ dihydrate. Excess, loosely bound moisture was still present.

Table 6.17: Overview of inputs and results for the oven drying experiment at 40°C, 20% RH and 0.20 bar – CBZ dihydrate.

40°C, 20% RH and 0.20 bar – CBZ dihydrate	
<i>Post drying</i>	
<i>Av. End point M.C. (%)</i>	17.03±0.36
<i>Desired M.C. (%)</i>	13.22

While the lower pressure allowed for the experimental temperature to remain constant, the model underpredicted the drying time at these conditions. Further work was needed to understand the inability of the model to accurately predict drying time when a higher temperature was used. This further work involved updated calculations which are discussed in **Section 6.5.4**.

6.5.3.2. THEO Monohydrate

6.5.3.2.1. Drying at 30°C, 20% RH and 0.20 bar

An analogous set of experiments as discussed in **Section 6.5.3.1** for CBZ were carried out on portions of THEO monohydrate. A batch of wet THEO monohydrate was generated as per the method detailed in **Section 2.3.1.8** and the sample was prepared for oven drying as per **Section 2.10.1.3**. **Figure 6.12** shows a screenshot of the model, via Excel, being used to predict the drying time of this experiment.

The experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.18** below:

Table 6.18: Conditions and inputs for the oven drying experiment at 30°C, 20% RH and 0.20 bar – THEO monohydrate.

	30°C, 20% RH and 0.20 bar
T_g (°C)	30.00
Measured outside RH (%)	20.00
Oven Pressure (bar)	0.20
Pre-drying MC (%)	40.00
Initial sample weight (g)	1.02569
X*	0.00

Experiment 253						
T _g (°C)	30	User input	Total Drying time (min)			
Measured outside RH (%)	20		Predicted	59.39		
Oven Pressure (bar)	0.2					
Initial weight (g)	1.02569		Prediction			
Pre drying MC (%)	40.00		X	Time (s)	Time (min)	Time (h)
X* (-)	0.00		0.52	0	0	0.00
RH inside oven (%)	1.789191206		0.42	341.888	5.7	0.09
T wet bulb (°C)	10.24		0.32	683.776	11.4	0.19
Initial wet solid (g)	1.02569		0.22	1025.66	17.1	0.28
hydrate dry wt (g)	0.676954034		0.12	1368.72	22.8	0.38
Gas Temperature (K)	303.15		0.07	1610.46	26.8	0.45
Wet Bulb Temperature (K)	283.39		0.02	2229.51	37.2	0.62
Initial wet solid (kg)	1.03E-03		0.005155	2687.25	44.8	0.75
hydrate dry wt (kg)	6.77E-04		0.004155	2778.79	46.3	0.77
Initial moisture content (-)	0.52		0.003155	2895.67	48.3	0.80
Critical Moisture Content (X _c) (-)	0.124	From DVS	0.002155	3057.5	51.0	0.85
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	13.741	From DVS	0.001655	3169.57	52.8	0.88
Constant Drying rate (kgs ⁻¹)	1.98E-07		0.000655	3563.18	59.4	0.99
MW THEO (g/mol)	180.164					
MW THEO-MH(g/mol)	198.18					
Theoretical wt of all moisture (g)	0.410276					
Theoretical endpoint wt (Anhy/dehy) (g)	0.615414					
Theoretical wt of dry monohydrate (g)	0.676954034					

Figure 6.12: Input section of model predicting the drying time for the oven drying experiment for THEO monohydrate at 30°C, 20% RH and 0.20 bar.

Using the same updated calculations as detailed in **Section 6.5.2**, the predicted drying time for this sample was 59 min. Immediately after the experiment (after 59 min), % Moisture Content value was obtained using the Moisture Analyzer (**Section 2.5.2**). This analysis was completed singularly and showed that the end point sample had a % Moisture Content value of 9.92% which is only slightly greater than the expected value of 9.08% for THEO monohydrate,

Table 6.19. A minimal amount of excess, loosely bound moisture is suspected to be present with the monohydrate. XRPD analysis was not carried out on this sample as it was misplaced prior to analysis.

Table 6.19: Overview of inputs and results for the oven drying experiment at 30°C, 20% RH and 0.20 bar - THEO monohydrate.

30°C, 20% RH and 0.20 bar – THEO monohydrate	
<i>Post drying</i>	
<i>Av. End point M.C. (%)</i>	9.92
<i>Desired M.C. (%)</i>	9.08

The model appears to have worked in predicting the drying time for THEO monohydrate at these conditions. When compared to the same experiment on CBZ dihydrate, **Section 6.5.3.1.1**, the model underpredicted slightly for THEO monohydrate which is more preferable to the slight over-drying seen with the CBZ dihydrate sample. The main aim of this entire work is to establish a drying protocol where the dry hydrate form is produced and ultimately the hydrate form is not negatively impacted by the drying method.

6.5.3.2.2. Drying at 40°C, 20% RH and 0.20 bar

The second experiment on THEO monohydrate was set up using the same preparation methods previously discussed. **Figure 6.13** shows a screenshot of the model, via Excel, being used to predict the drying time of this experiment. The experiment was set up as detailed in **Section 2.10.1.3** with the inputs detailed in **Table 6.20** below:

Table 6.20: Conditions and inputs for the oven drying experiment at 40°C, 20% RH and 0.20 bar - THEO monohydrate.

	40°C, 20% RH and 0.20 bar
T_g (°C)	40.00

Measured outside RH (%)	20.00
Oven Pressure (bar)	0.20
Pre-drying MC (%)	23.23
Initial sample weight (g)	1.01665
X*	0.00

Experiment 254						
T _g (C)	40	User input	Total Drying time (min)			
Measured outside RH (%)	20		Predicted		40.05	
Oven Pressure (bar)	0.2					
Initial weight (g)	1.01665		Prediction			
Pre drying MC (%)	23.23		X	Time (s)	Time (min)	Time (h)
X* (-)	0.00	0.18	0	0	0.00	
RH inside oven (%)	0.82826747	0.08	367.973	6.1	0.10	
T wet bulb (C)	14.79	0.07	421.337	7.0	0.12	
Initial wet solid (g)	1.01665	0.06	482.44	8.0	0.13	
hydrate dry wt (g)	0.858528693	0.05	553.913	9.2	0.15	
Gas Temperature (K)	313.15	0.04	640.014	10.7	0.18	
Wet Bulb Temperature (K)	287.94	0.03	748.308	12.5	0.21	
Initial wet solid (kg)	1.02E-03	0.02	894.368	14.9	0.25	
hydrate dry wt (kg)	8.59E-04	0.01	1119.6	18.7	0.31	
Initial moisture content (-)	0.18	0.00418	1635.23	27.3	0.45	
Critical Moisture Content (X _c) (-)	0.124	From DVS	0.00218	1910.19	31.8	0.53
Convective heat transfer coefficient (h) (Wm ⁻² K ⁻¹)	13.741	From DVS	0.00168	2020.29	33.7	0.56
Constant Drying rate (kgs ⁻¹)	2.53E-07		0.00068	2403.01	40.1	0.67
MW THEO (g/mol)	180.164					
MW THEO-MH(g/mol)	198.18					
Theoretical wt of all moisture (g)	0.236167795					
Theoretical endpoint wt (Anhy/dehy) (g)	0.780482205					
Theoretical wt of dry monohydrate (g)	0.858528693					

Figure 6.13: Input section of model predicting the drying time for the oven drying experiment for THEO monohydrate at 40°C, 20% RH and 0.20 bar.

Using the same updated calculations, the predicted drying time was 40 min. % Moisture Content analysis was collected for a single sample and found that the end point sample (after 40 min drying) had a % Moisture Content value of 8.53% which is slightly lower than the expected value of 9.08% for THEO monohydrate, **Table 6.21**. XRPD analysis of the sample, was overlaid with the monohydrate form and is shown in **Figure 6.14**. Results here show that the monohydrate form is still present. To determine the level of water present more accurately, TGA analysis was carried out on the end point sample (**Figure 6.15**). TGA gave a value close to the

Moisture Analyzer value, at 8.877%. Despite both values being marginally lower than expected, XRPD results show the monohydrate was still present after being dried for the predicted amount of time.

Table 6.21: Overview of inputs and results for the oven drying experiment at 40°C, 20% RH and 0.20 bar - THEO monohydrate.

40°C, 20% RH and 0.20 bar – THEO monohydrate	
<i>Post drying</i>	
<i>Av. End point M.C. (%)</i>	8.53
<i>Desired M.C. (%)</i>	9.08

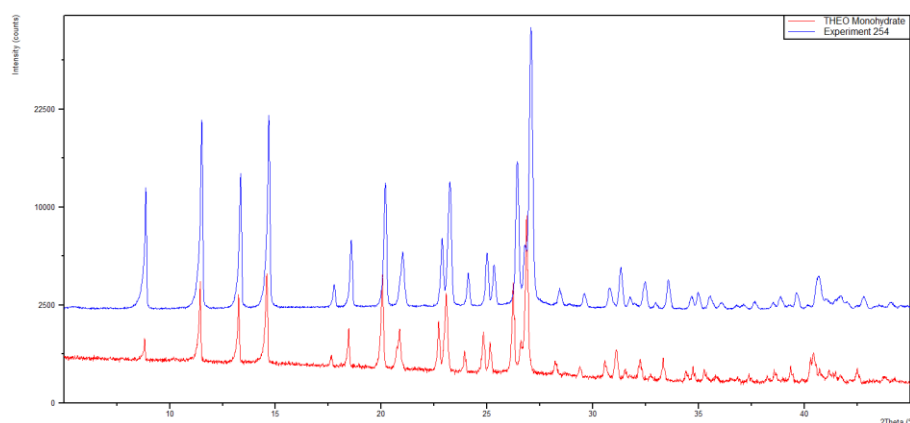


Figure 6.14: XRPD overlay of THEO monohydrate (red) and the end point sample (blue) of the experiment at 40°C, 20% RH and 0.20 bar.

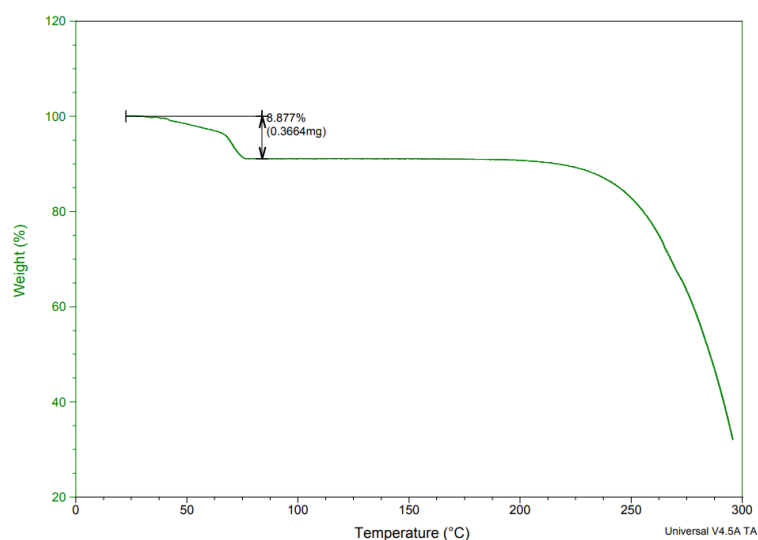


Figure 6.15: TGA analysis of the end point sample of the experiment at 40°C, 20% RH and 0.20 bar.

Again, the model appears to have correctly predicted the drying time for THEO monohydrate at this higher temperature which is different to the results obtained for the same experiment carried out on CBZ dihydrate in **Section 6.5.3.1.2**. At the higher temperature, the model underpredicted for the CBZ dihydrate sample with ~4% excess, loosely bound moisture still being present at the predicted drying time for the dry hydrate. The differences seen between the two compounds within these experiments led to some final experiments to gather more drying data within the constant drying rate period to help determine how far out the model prediction was to the experimental data.

6.5.4. Constant rate period validation experiments

The final update to the model involved an updated equation for the wet bulb temperature calculation. All predicted drying times up to this point were made using Eq. 29 for the calculation of T_w . After which, the T_w value was incorporated into Eq. 26-28 to give the predicted drying time.

In this final section, T_w was calculated using Eq. 24, which relates the T_w value to the absolute humidity, using the Antoine equation to calculate the % RH inside the oven. The relationship between T_w and the absolute humidity is provided by a mass/enthalpy balance using a heat transfer coefficient (h_G) and a mass transfer coefficient (k_Y). As the experiments carried out are for a single solvent (water) system, for air-water mixtures the ratio $\frac{h_G}{k_Y}$ (in Eq. 24) can be taken to be 950 N.m/kg.K. ^[126] The updated T_w value from Eq. 24 then feeds into Eq. 29-31, as detailed in **Section 6.4.2**.

This updated model was tested with a triplicate of drying experiments, each drying a portion of CBZ dihydrate at 30 °C in the oven, and 20% RH (outside). Each sample came from the same bulk cake and all experiments were carried out on the same day; therefore, the same 'Pre-drying %M.C. (%)' value is carried out through each experiment.

As T_w is used to calculate the constant rate period (Eq. 29) prior to the predicted drying time, it was decided to stop each of the three experiments during the suspected constant drying rate period to determine how close or far from the experimental values the updated model predictions now compared.

6.5.4.1. CBZ Dihydrate – (1) 30°C, 20% RH and 0.20 bar

Table 6.22 shows an overview of the user inputs and sample details for the first experiment using the updated model. As previously mentioned, each of these experiments was to be stopped during the constant drying rate period. For this experiment, 11 min was estimated to be within the constant rate period based on 'Predicted vs. Experimental' graphs produced within the Excel version of the model. The actual predicted drying time for this sample was 46 min.

It should be noted that an experiment with the same conditions was carried out in **Section 6.5.3.1.1** and it had a much longer drying time, at 87 min. While the updated calculation for T_w used here would change the drying time, its effect would not be expected to be this large. The difference in drying time here is influenced more by the larger sample sized (1.02 g) and the larger initial % moisture content value (62.50%) for the sample in **Section 6.5.3.1.1**. compared to the sample studied here (sample size 0.74 g and moisture content 50%).

Figure 6.16 shows the ‘Predicted vs. Experimental’ curve (moisture content vs. time) for the experimental data (from **Table 6.23**). The % moisture content value is the total moisture in the sample, which included both the loosely bound and the water of hydration. It was required to calculate a moisture content value based on the wet hydrate, i.e., loosely bound moisture on the hydrate, this is denoted by M.C. (-). This is done using the calculations below:

M.C. (-) =

$$\frac{\text{Initial weight of sample to be dried (g)} - (\text{dry weight of hydrate (g)} * 1000)}{\text{dry weight of hydrate (g)} * 1000}$$

Eq. 34

Where:

Weight of dry hydrate =

$$\frac{\text{Theoretical end point weight of sample}}{\text{MW of anhydrate}} \times \text{MW of hydrate} \quad \text{Eq. 35}$$

Theoretical end point weight =

$$\text{Initial weight} - \text{theoretical weight of all moisture} \quad \text{Eq. 36}$$

$$\text{Theoretical weight of moisture} = \left(\frac{\text{Initial weight}}{100} \right) * \text{Pre drying \% M.C.} \quad \text{Eq. 37}$$

After 11 min, the sample contained 35.04% moisture content, compared to the updated prediction made by the model of 32.10% moisture content at the same timepoint. While the model underpredicts, a difference in moisture content of only ~3%, shows good comparability of the model to the experimental data. The underprediction could be due to fluctuations in the oven in terms of pressure, temperature, and humidity causing the actual drying to be slower than what the prediction states. When compared to the DVS, the oven has less control on these parameters.

Table 6.22: Overview of inputs for the final version of the model for the CBZ dihydrate oven drying experiment (1) at 30°C, 20% RH and 0.20 bar.

(1) 30°C, 20% RH and 0.20 bar – CBZ dihydrate	
<i>Model inputs</i>	
<i>T_g (°C)</i>	30
<i>Measured outside RH (%)</i>	20
<i>Oven Pressure (bar)</i>	0.20
<i>Initial weight (g)</i>	0.7414
<i>Pre drying M.C. (%)</i>	50.00
<i>X* (-)</i>	0.00
<i>Wet bulb temperature (°C)**</i>	8.31

* An Assumption carried through from the DVS drying studies in Chapter X.

** Calculated separately, then used as an input.

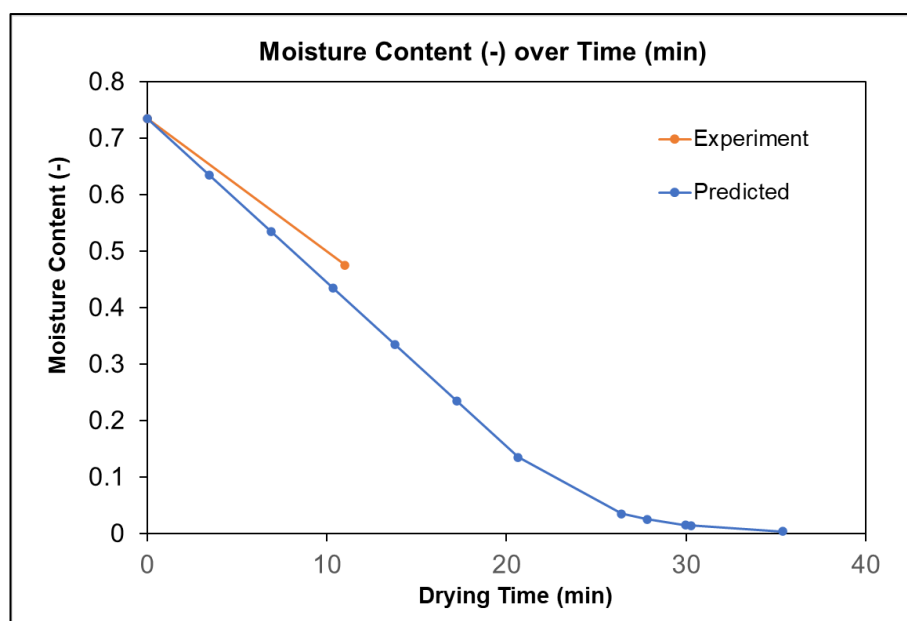


Figure 6.16: Moisture content (-) versus Time (min) for CBZ dihydrate oven drying at 30 °C, 20% RH and 0.20 bar (experiment 1).

Table 6.23: Comparison of start and end results for the CBZ dihydrate oven drying experiment (1) at 30°C, 20% RH and 0.20 bar.

(1) 30°C, 20% RH and 0.20 bar – CBZ dihydrate			
<i>Time (min)</i>	<i>M.C. (%)</i>	<i>Mass (g)</i>	<i>M.C. (-)</i>
0	50.00	0.7414	0.735
11	35.04	0.6305	0.476

6.5.4.2. CBZ Dihydrate – (2) 30 °C, 20% RH and 0.20 bar

Table 6.24 shows the user inputs and sample details for the second experiment at 30 °C, 20% RH and 0.20 bar. The only difference to the first experiment is the initial sample weight, which is slightly increased from 0.7414 g to 0.8278g. This experiment was stopped after 13 min as this was believed to be within the constant rate period, like the previous experiment. The additional time compared to the previous experiment is due to the extra sample weight as all other parameters remained the same. The predicted drying time

for this sample was 51 min. As mentioned in the previous section, the difference in predicted drying time for this sample versus the prediction in **Section 6.5.3.1.1**, is due the increased sample sized (1.02 g) and the larger initial % moisture content value (62.50%) of the previous sample.

The 'Predicted vs. Experimental' curve (moisture content vs. time), shown in **Figure 6.17**, is based on the experimental data from **Table 6.25**. After 13 min, the sample contained 27.51% moisture content while the predicted value at the same timepoint was calculated to be ~30.95%. For the experiment, the model appears to have overpredicted the % M.C. of the sample at this timepoint, with a ~3.5% difference seen. While the values here do compare well, when the model overpredicts how much moisture will be lost within the drying process, it would lead to a longer drying time. This is not a favourable outcome as the experimental data suggests the drying process is occurring faster. If the drying experiment had been continued to completion, based on the prediction, the water of hydration may have been negatively impacted, as it would have been dried longer than required.

When compared to the previous experiment, an extra ~8% M.C. was removed in this experiment. Uniform drying across a cake is very difficult to determine but all efforts were made to ensure sample cakes were prepared similarly to each other to minimise differences. A thinner layer of sample cake could be the reason behind more moisture being removed. Issues with parameter control could also be playing a role in the drying process occurring faster for the experiment.

Table 6.24: Overview of inputs for the model for the final version of the model for the CBZ dihydrate oven drying experiment (2) at 30 °C, 20% RH and 0.20 bar.

(2) 30 °C, 20% RH and 0.20 bar – CBZ dihydrate	
Model inputs and sample details	
T_g (°C)	30
Measured outside RH (%)	20
Oven Pressure (bar)	0.20
Initial weight (g)	0.8278
Pre drying M.C. (%)	50.00
X^* (-)	0.00
Wet bulb temperature (°C)**	8.31

* An Assumption carried through from the DVS drying studies in Chapter X.

** Calculated separately, then used as an input.

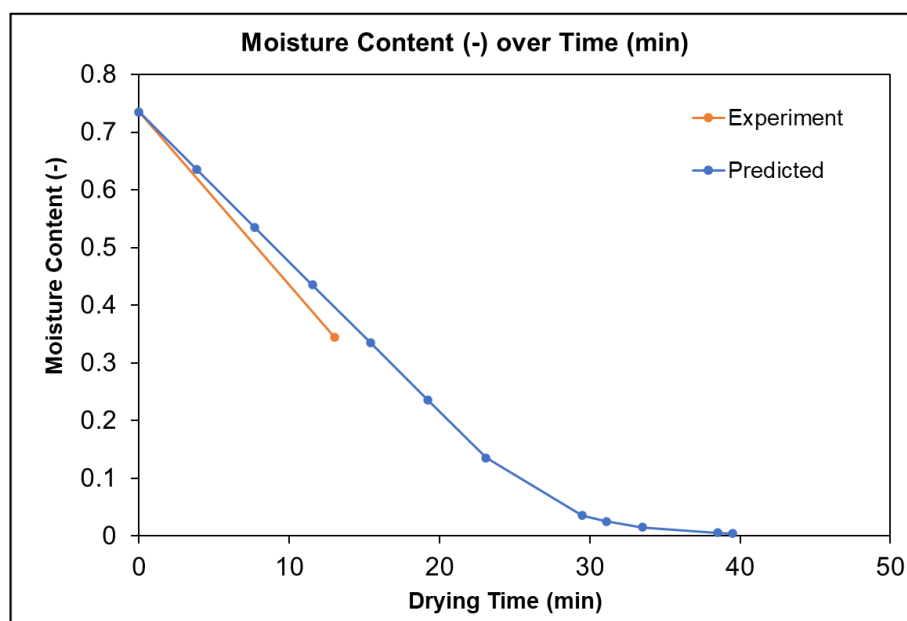


Figure 6.17: Moisture content (-) versus Time (min) for CBZ dihydrate oven drying at 30 °C, 20% RH and 0.20 bar (experiment 2).

Table 6.25: Comparison of start and end results for the CBZ dihydrate oven drying experiment (2) at 30 °C, 20% RH and 0.20 bar.

(2) 30 °C, 20% RH and 0.20 bar – CBZ dihydrate			
<i>Time (min)</i>	<i>M.C. (%)</i>	<i>Mass (g)</i>	<i>M.C. (-)</i>
0	50.00	0.7414	7.35E-01
13	27.51	0.6416	3.45E-01

6.5.4.3. CBZ Dihydrate – (3) 30 °C, 20% RH and 0.20 bar

The user inputs and sample details for the third experiment are shown in **Table 6.26**. Another slight increase in sample weight, from 0.8278 g to 0.8882 g, was the only change in this experiment relative to the previous one. This experiment was stopped after 16 min to fall with the constant rate period. The additional time once again is due to the increase in sample weight as all other parameters remain the same. The predicted total drying time for this sample was 55 min.

The ‘Predicted vs. Experimental’ curve (moisture content vs. time), shown in, **Figure 6.18**, using the experimental data from **Table 6.27**. After stopping the experiment at 16 min, the sample contained 24.47% moisture content which was less than the predicted value of ~28.35% at the same timepoint. Like the previous experiment, the model overpredicted the % M.C. at this timepoint, with a difference of ~3.80% seen. This compares well to the previous experiment but differs to the first. As mentioned for the last experiment, the model overpredicting the % M.C. throughout the drying process is less favourable as it leads to longer drying times. For the first experiment, if the trend had continued with the model underpredicting the amount of moisture present, the sample obtained at the end may still contain a small amount of loosely bound moisture, but the hydrate would be preserved. That would not be the case for the samples obtained from the final two experiments. Using specific conditions, like a % RH inside the oven of $\geq 40\%$ RH, based on DVS data, could provide an environment

where hydrate stability was likely and this overprediction of ~3% may not be enough to jeopardise the hydrate but ideally, the model would not overpredict the time required for drying. Running a set of experiments to completion would give a better insight into how this underprediction plays out throughout a full drying process.

Once again, when compared to the previous experiment, **Section 6.5.4.2**, slightly more moisture was removed despite an increase in sample size. The issue of uniform drying across the cake and parameter control are again issues to contend with but is difficult to determine and control within the oven.

Table 6.26: Overview of inputs for the model for the final version of the model for the CBZ dihydrate oven drying experiment (3) 30 °C, 20% RH and 0.20 bar.

(3) 30 °C, 20% RH and 0.20 bar – CBZ dihydrate	
<i>Model inputs and sample details</i>	
<i>T_g (°C)</i>	30
<i>Measured outside RH (%)</i>	20
<i>Oven Pressure (bar)</i>	0.20
<i>Initial weight (g)</i>	0.8882
<i>Pre drying M.C. (%)</i>	50.00
<i>X* (-)</i>	0.00
<i>Wet bulb temperature (°C)**</i>	8.31

* An Assumption carried through from the DVS drying studies in Chapter X.

** Calculated separately, then used as an input.

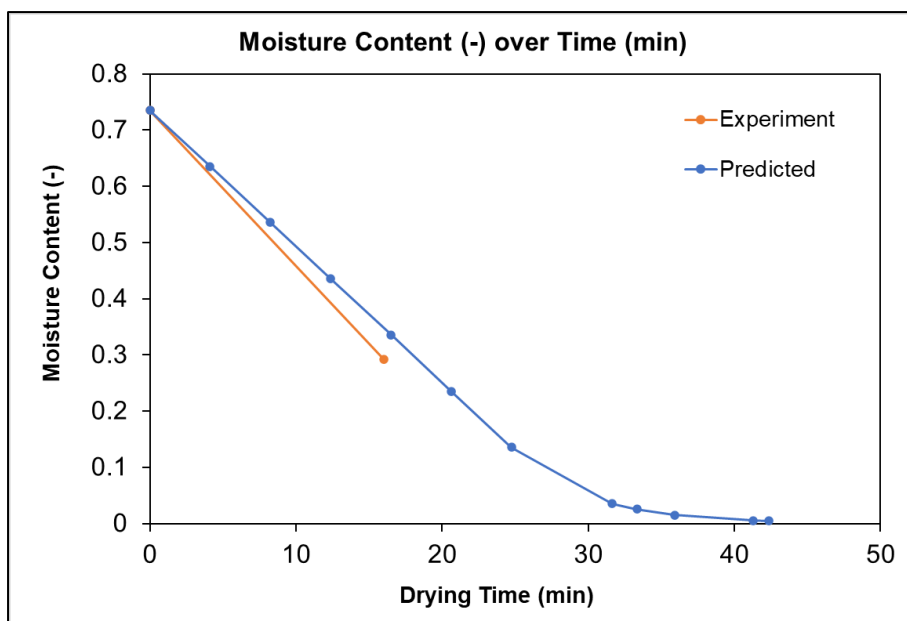


Figure 6.18: Moisture content (-) versus Time (min) for CBZ dihydrate oven drying at 30 °C, 20% RH and 0.20 bar (experiment 3).

Table 6.27: Comparison of start and end results for the CBZ dihydrate oven drying experiment (3) at 30 °C, 20% RH and 0.20 bar.

(3) 30 °C, 20% RH and 0.20 bar – CBZ dihydrate			
<i>Time (min)</i>	<i>M.C. (%)</i>	<i>Mass (g)</i>	<i>M.C. (-)</i>
0	50.00	0.7414	7.35E-01
16	24.47	0.6614	2.92E-01

While the triplicate set of experiments showed differences, with one underpredicting and the other two overpredicting, the difference between the model and experimental data was <5% in each case. The highly controlled environment of the DVS has proved very difficult to replicate on a larger scale and is likely the cause for the small amounts of loosely bound moisture still being present.

6.6. Chapter Conclusions

Development work on the drying model saw new versions incorporate calculations for % RH inside the oven, vapour pressure values, the Antoine equation and an updated calculation for the wet bulb temperature which helped to fully describe the environment inside the oven based on both the oven temperature and the oven pressure. Before these updates, early versions of the model significantly underpredicted the drying times of both hydrate compounds. By using and combining the Antoine equation and the latest T_w calculation, it allowed for the relationship between vapour pressure and temperature to be understood and incorporated into the model calculations of % Moisture Content over Time.

The DVS experimental results were successfully used to determine the critical moisture content (X_C) and convective heat transfer coefficient (h_C). Coupling the final version of the model with its three constant rate period validation experiments, results showed the model was both under and over predicting the drying times with differences in predicted and experimental moisture contents varying by <5%. There are many variables in the oven when compared to the DVS which is controlled to a lesser extent, e.g., pressure, temperature and % RH. The ability to vary the critical moisture content based on calculations from the DVS data, highlights the sensitivity of model and this proved important when predicting the oven drying times at larger scales.

Further opportunities for developing the platform include using the latest model to run a set of full-length experiments to confirm whether the undesired overprediction of drying time in **Sections 6.5.4.2** and **6.5.4.3**, is still a factor, and if so, to what extent. The use of longer experimental runs (e.g., with wetter sample cakes)

could indicate whether errors or simplifications associated with the model are compounded over longer timeframes, or whether difficulties associated with parameter control in the oven are the leading cause of the discrepancy between predicted and experimental drying profiles. Then an investigation of data sets in which organic solvates are examined in place of hydrates, thus allowing for data collection for a wide range of compounds would be useful, using this data for calculating X_C and h_C , and finally using this to predict the drying of solvates.

CHAPTER 7. General Conclusions

7.1. General discussion and conclusions

As outlined in the introduction chapter, the project aimed to study how drying conditions (temperature, % RH, pressure) affect the stability of hydrated model APIs and develop a consistent drying platform to produce the desired dry hydrate form. Drying is a complex process involving heat and mass transfer, influenced by factors like temperature, % RH, pressure, and gas flow rate. APIs are sensitive to drying stresses, and choosing the right temperature is crucial for product quality and drying times. High temperatures yield shorter drying times but may affect product quality, while lower temperatures preserve quality but increase drying time and cost. Accurate drying time prediction is valuable for cost estimation and energy consumption management.

In the first results chapter, **CHAPTER 3**, the main goal of this work was to build a library of solid-state results for both model compounds, CBZ and THEO, their hydrate forms, and polymorphs. A further goal was to generate batches of hydrate crystals with differing particle sizes. Firstly, establishing robust methods for hydrate generation was the key objective in this work. CBZ dihydrate was successfully generated using a slurry conversion method with DIW. The transformation from the anhydrous to dihydrate was found to occur faster than expected, with most of the small, irregular crystals quickly transforming to needle-like crystals of the hydrate form within the first hour. THEO monohydrate was generated using a cooling crystallisation method. Both methods were carried out in purely aqueous solutions, meaning future drying work involved air-water systems only. Dealing with a single solvent system was advantageous with respect to fully understanding the drying process.

Generation of each of the polymorphs based on methods from the literature proved more difficult. For CBZ, anhydrous Form I and II polymorphs were successfully generated. The methods used ranged from crystallisations from ethanol for Form II and direct heating of a solid with overhead agitation for Form I. However, attempts to produce Form IV, from an ethanol crystallisation process, failed, giving only a mixture of the Form III starting material and Form IV. Issues were also encountered in generating the THEO polymorphs. The Form III polymorph was successfully generated. However, attempts to generate Form I and IV were unsuccessful, with both products containing a mixture of the Form II starting material and the monohydrate. Nevertheless, the lack of solid-state data on the unsuccessfully generated forms did not hinder future work, and a library of results was collected which would aid form identification in post drying experiments.

Solubility data was then collected for both hydrate forms of the model compounds. Collecting solubility data of a compound is the first step in developing a crystallisation process and it was hoped that by collecting this data, methods could be developed for generating hydrated crystals of varying particle size. For CBZ dihydrate, a few methods for collecting solubility data were attempted, involving the use of Crystal 16™ and FBRM, but it was clear from failed attempts that CBZ dihydrate was very poorly soluble in water. Due to this, a gravimetric method was employed. This method is known to be a very accurate option for poorly soluble materials; however, it is a lengthy and tedious process in comparison to the more automated options available. As expected, data showed the hydrate form was very poorly soluble in water. THEO monohydrate proved a much easier compound for which to collect solubility using Crystal 16™, and cloud and clear points were easy to determine. THEO monohydrate had a higher solubility when compared to CBZ dihydrate. Where possible, these findings informed methods for generating crystals of differing PSD. Due to the fast transformation of anhydrous CBZ to CBZ dihydrate when added to water, using a crystallisation process (e.g., rapid cooling crystallisation) to generate small crystals was unlikely to be successful, so milling was used to downsize CBZ dihydrate crystals generated in the standard crystallisation method. Despite the limited solubility of CBZ dihydrate, large crystals were produced by utilizing a temperature cycling technique, by using an upper temperature that had no negative effects on the hydrate form (<80 °C). Both for the robust generation of monohydrate crystals as well as the manufacturing of hydrate crystals with different particle sizes, cooling crystallization methods turned out to be the most effective approach for THEO monohydrate crystals.

This work successfully led to the development of reliable methods for producing hydrate forms for use throughout the project. While issues were experienced trying to generate the polymorphs for each of the compounds, later work was not affected by the lack of this data. The successful generation of crystals of differing PSD allowed for the next step of the project to begin: to examine how micromeritic characteristics affected hydrate stability and drying.

The next results chapter, **CHAPTER 4**, investigated hydrate stability using the Cuspor Aging System. The objective of this work was to determine the time taken for CBZ dihydrate and THEO monohydrate to lose their water of hydration and show evidence of a form change to an anhydrous or dehydrated form, under controlled temperature and % RH conditions. A further objective was to determine if the hydrate forms remained stable under any set of conditions employed. It was hoped that results generated here would provide information on form boundaries and give initial insights into the design space within which the hydrates could be successfully dried, while retaining the hydrate form, allowing for future drying experiments to come from a place of knowledge and to be data driven.

Downsides which were identified to using this equipment setup were, not only the invasive sampling required, which involved opening each vial and disturbing the environment, but also the significant sample preparation and the amount of sample required. Based on the sampling plan that was set out for this work, it was decided that using a cross section of conditions was best course of action. For both CBZ dihydrate and THEO monohydrate, evidence of hydrate stability was only found under one set of conditions employed: 75% RH and 25 °C. Under these conditions, hydrate stability was shown for both compounds over a 12-week timeframe. At the other conditions, the hydrate forms were

negatively affected, with loss of the hydrated forms and transition back to the anhydrous starting materials (i.e., anhydrous forms from which the hydrate was originally generated). An exception was seen for one sample of CBZ dihydrate, held at 2% RH and 25°C, for which a transition to a mixture of forms was observed, with the XRPD overlay showing mostly the Form III starting material being present, but also characteristic peaks from Form I (at 9.40° 2θ) and the dihydrate (at 12° 2θ) being present. The presence of Form I at these conditions was expected, based on a method of forming Form I crystals from literature ^[75] which found that dehydrating the dihydrate under 5-60% RH was successful in producing Form I. This previous work detailed the use of a slightly higher temperature range of 30-50°C, which may be the reason behind the mostly Form III mixture in the current work. Both model compounds were found to be negatively impacted by low % RHs and high temperatures. An environmental condition of 2% RH was too low for hydrate stability to be achieved, regardless of temperature, and at 50 °C and 80 °C, regardless of % RH used, the water of hydration was lost.

To examine the effects of micromeritic properties on dehydration, hydrated crystals of differing PSD (generated as previously discussed) were analysed in the same stability study. For CBZ dihydrate, differences due to PSD were found at two sets of conditions: 75% RH/25 °C and 40% RH/50 °C. While TGA findings showed moisture levels corresponding to the hydrate form was present across all three samples at 75% RH and 25 °C over the 12 weeks, XRPD results showed the beginning of Small and Large crystals shifting to the Form III CBZ anhydrous material, while Medium crystals preserved the dihydrate form for the whole 12-week period. Due to larger surface area of the small particles, as more of the sample is exposed to the environment, it could have been affected by the environmental conditions to a greater extent

or possibly, mechanically produced defects caused by the milling process, to achieve the smaller PSD, weakened the bonds holding the water within the crystal lattice reducing hydrate stability. For the larger particles, temperature cycling appears to have caused a reduction in hydrate stability in comparison to the Medium crystals. Thermocycling has been shown to both reduce phase transition temperatures and increase dislocation densities with a crystalline material. ^[127] A dislocation is an abrupt change in the arrangement of atoms inside a crystal structure causing a crystal defect. It is possible that an increase in these defects may have impacted the hydrogen bonding within the hydrate channels, allowing water to exit more readily. Even with these considerations, the stability of the hydrate form at these conditions is significant. For the centre point conditions of 40% RH/50 °C, water of hydration was lost faster across all three sample (Small, Medium, and Large) in comparison to the previously discussed conditions of 75% RH/25°C. After one week, Small and Medium crystals contained no water of hydration whereas Large crystals still contained ~1% moisture at this timepoint. Even though a difference due to PSD was shown here, the hydrate form was not preserved in any case. Samples of THEO monohydrate with differing PSD showed no differences in dehydration behaviour under different stability conditions. The loss of the hydrate form resulted in all samples transitioning back to the anhydrous Form II starting material. Each sample reacted the same way; where the hydrate form was lost, it was lost across the Small, Medium, and Large crystals. The same is true of the condition in which the monohydrate stayed stable (75% RH/25°C), the hydrate form was seen in each sample of differing PSD. Although the research revealed commonalities between the two compounds, form boundaries are unique to each molecule, and establishing a drying procedure requires an understanding of the hydrate's stability and

its relationship to the anhydrous forms. From this stability study, findings showed that temperature and % RH levels had an impact on the hydrate's stability. These preliminary findings, while limited, were still useful for informing future drying experiments.

A previously mentioned downside, regarding the setup of this study was the invasive sampling approach, which caused changes to the testing environments during every sampling point, meaning the specific conditions, under which the sample was being tested, had to equilibrate after each sampling point. This could, in turn, affect the dehydration profile and the true time taken for these transitions. To access real-time, uninterrupted data it was decided to use the DVS to examine both the drying (loss of loosely bound moisture) and dehydration (loss of water of hydration) processes.

This instrument can track changes in the sample weight in real time, while maintaining a highly controlled environment. It also generates a small amount of sample at the conclusion of each experiment, allowing for off-line solid-state analysis and additional data to be gathered. Moving studies to the DVS, proved to be one of the most impactful findings of this project. In **CHAPTER 5**, for the two model hydrate compounds, it was demonstrated that the DVS could be successfully employed for small-scale drying studies. It offered a highly controlled environment and captured data every minute, enabling the collection of drying and dehydration kinetics data as well as the plotting of repeatable drying curves with many data points. Initial experiments, at 0% RH and 40°C were carried out knowing both drying and full dehydration would take place. As a result, a graph of moisture content versus time was generated, with sections of the curve separated by an inflection point. It was later confirmed that the point of inflection did denote changes in the type of moisture being removed. The stage before the point of inflection was related to the loss of excess, loosely bound moisture

and after this point, related to dehydration (i.e., the loss of water of hydration). This was a significant result related to one of the main objectives of this project, as the time point where the transition from drying to dehydration (or loss of stability of the hydrate) occurs could be pinpointed for both hydrate compounds, even at the harsh conditions of 0% RH and 40°C. Along with this, the data generated via the DVS allowed for drying rates to be calculated and traditional drying curves (drying rate vs. time) to be graphed. From one experiment, the potential of the DVS to be used as a small-scale drying screening tool became clear.

Experiments were conducted on both hydrate forms using the DVS to understand its capabilities and limitations. They explored temperature, % RH, PSD, and increased sample size effects on drying, hydrate stability, and dehydration. The multiple step method allowed data collection for drying, hydrate stability, and dehydration within the same experiment.

Analysis of the drying of CBZ dihydrate showed that under conditions of increasing % RH, the time taken for the removal of excess, loosely bound moisture increased, and increasing the temperature reduced drying times. Both were expected results. Once the loosely bound moisture was removed, the stability of the dry dihydrate was found in environments $\geq 40\%$ RH. In lower % RH environments, hydrate stability was < 10 min and the possibility for failure in obtaining the hydrate form consistently is much higher at lower % RH conditions. Dehydration immediately followed the short-lived period of stability and often with all water of hydration being fully removed prior to the 0% RH step being implemented. For the higher RH values, dehydration was induced only when the 0% RH period began, and dehydration of these samples consistently took approximately 30 mins for the $\sim 13\%$ moisture to be removed. The end point samples of those analysed at low % RH

showed that the original anhydrous Form III starting material remained post DVS “drying”. For higher % RH samples, analysis of the end point samples indicated differences in the crystal structure between the dehydrated CBZ, the dihydrate form and the anhydrous Form III starting material. The use of non-ambient XRPD measurements showed the transition from CBZ dihydrate to an amorphous form during drying and this is believed to be caused by the breaking of the amide C=O bond ^[118], followed by the rearrangement of the lattice to give the Form III starting material. It is possible that the differences seen in the end point sample XRPD traces is due to this rearrangement within the lattice.

Investigating the effects of PSD, at 40% RH and 40°C (with respect to removal of loosely bound moisture), the Large crystals dried fastest, which was an unexpected and unexplained result. Small and Medium crystals had very similar drying times which was likely due to both batches of sample having similar d10 and d50 values. Post drying, hydrate stability was only seen for the Medium crystals, while slow and gradual dehydration was evident for Small and Large crystals, as the sample weight slowly decreased over time after all loosely bound moisture was removed. This finding corresponds to a similar result seen in the stability study (in the Cuspor system), where the stability of CBZ dihydrate, at 75% RH and 25°C, was confirmed at the 12-week time point for Medium crystals, while the Small and Large crystals showed the start of a transition to the anhydrous Form III starting material. In the DVS, dehydration was induced for each sample by implementing the 0% RH step. Small and Medium crystals took a similar amount of time to fully dehydrate while Large crystals had the longest dehydration time. Longer channels are the probable cause of this, with more time required for the water to move through the channels and be released.

Increasing the sample size in the DVS more than doubled the drying time. The cake depth of this sample was much greater than the standard DVS sample because the dihydrate tended to clump while kept damp, and this increase in migration distance which the moisture had to travel would have a significant impact in the drying time.

THEO monohydrate results showed that an increase in % RH resulted in longer drying times and that increased temperatures gave shorter drying times, as was seen with CBZ dihydrate. Significant periods of dry hydrate stability were seen for THEO monohydrate samples at 40% RH and above, with stability only being negatively impacted when the 0% RH step began, causing dehydration. At 0 and 10 % RH levels, only a point of inflection detailed the end of the drying process and the start of dehydration. At 20% RH, a short ~10 min period of hydrate stability preceded dehydration. Like CBZ dihydrate, these low levels of % RH would not provide a suitable environment for a robust drying method, as the boundaries between success and failure are very close.

In a more expected set of results (compared to the CBZ findings), when examining the effect of PSD, it was shown that drying times increased with PSD. The greater surface area of the Small crystals exposed to the drying environment led to shorter drying times. Hydrate stability was seen across each of the samples up to the beginning of the 0% RH step, after which dehydration began. Here, the time taken for % moisture content to stabilise was similar across each of the samples and took ~120 min. In comparison to CBZ dihydrate, dehydration took longer for THEO monohydrate. Activation energies of dehydration are significantly impacted by particle size, sample weight, crystal defects and intermolecular forces. ^[94] From **Sections 5.3.1.3** and **5.3.2.3**, the particle size analysis, across d10, d50 and d90 values showed THEO

monohydrate to have larger crystals than CBZ, which would contribute to larger hydrate channels. Water escaping the crystals is influenced by the size, shape, number of channels, hydrogen bonds with the host drug molecules, and both the quantity and strength of those bonds. ^[128] In the CBZ dihydrate structure, water molecules form two infinite parallel chains that are joined by hydrogen bonds. Additionally, the amide C=O and NH₂ groups of the CBZ molecules make alternate hydrogen bonds with the water molecules of each parallel chain. Its dehydration is determined by two overlapping processes, each of which occur at a different rate, and is strongly influenced by the environment. The fast process involves the removal of the less strongly bound water molecules attached to the amide C=O, and the slower process involves the removal of the more strongly bound water molecules attached to the amide N-H group. ^[118] In comparison, for THEO monohydrate, through two hydrogen bonds in the monohydrate structure, two theophylline molecules with centrosymmetrical relationships produce a dimer. The water molecules create infinite hydrogen-bonded chains that run along the a-axis in channels. Theophylline dimers act as hydrogen bonding agents to crosslink these parallel chains. As a result, parallel to the ab plane, two-dimensional hydrogen bound layers are created. ^[87] While both compounds involve parallel chains, the chains of THEO monohydrate are also crosslinked which may require more energy and time to break, leading to its longer dehydration time.

After dehydration of the monohydrate, the original anhydrous Form II starting material of THEO was present after drying under all conditions using the DVS. Non-ambient XRPD analysis carried out on THEO monohydrate showed a change from monohydrate to its metastable anhydrate once loosely bound moisture was removed. The metastable anhydrate is known to transition to the Form II

starting material ^[119] and while this wasn't shown through the non-ambient analysis, end point samples post DVS drying experiments, showed only the Form II starting material to be present in each sample. It is possible that during DVS analysis, the samples transitioned from the monohydrate to the metastable anhydrate and finally transitioned to the Form II starting material.

Increasing the sample size increased the drying time by only ~3 min. The THEO monohydrate damp cake does not clump like CBZ dihydrate and, while the sample size was increased, the cake depth was less than that of the CBZ dihydrate "larger" sample, which could be the reason behind the differences in drying times for both.

Overall, this small-scale drying study proved very successful, and, with this data, a large amount of information can be collected. Drying rates, % moisture content over time, drying curves, the timeframes where the dry hydrate forms are present and information on how long the hydrate forms remained stable were all determined. Based on these findings, and the large amount of data collected, work then began on the development of a predictive drying model. To develop a model for predicting drying times, a compound's drying rate curve data is required. Currently, this is mostly generated by sampling the cake at various time points along the drying curve, which provided information on the constant rate and falling rate periods. However, it can often be the case that post filtration, the wet cake is already below the critical moisture content, so the drying curve shows only the falling rate period. While removing as much unwanted moisture prior to drying is advantageous with respect to reducing drying times, full drying rate curve data is needed to build a robust drying model. Drying studies described in this project allowed for real time data to be collected throughout a highly controlled drying process. By using the DVS Intrinsic, comprehensive drying curves were generated and drying

kinetics data could be calculated. The experimental approach was also found to be suitable for samples with large amounts of excess, loosely bound moisture, eliminating the issues of the drying curves only containing the falling rate period.

The main aims of the work detailed **CHAPTER 6** included: (1) evaluating the feasibility of modelling the drying rate curve data for both CBZ dihydrate and THEO monohydrate using experimental DVS data that was previously generated, (2) where possible, model the DVS drying data for both compounds, to determine whether the modelling strategy can predict drying times while drying hydrate APIs in a convective drying environment, and finally, (3) scaling up the drying process to a lab-based oven and validating the model through scaled up experiments so that ultimately the model can be used to predict the drying times of both hydrate model compounds when dried in the oven. The first step in the model development was using DVS experimental results to predict the critical moisture content (X_c) and convective heat transfer coefficient (h_c). For oven drying, which was convective and for its predictive model, the X_c remains the same whereas the h_c must be scaled up to match the oven conditions.

The initial model significantly underestimated drying times for hydrate compounds. To improve the predictive model for oven drying, researchers had to understand the difference between setting a % RH on the outside of the oven and the actual % RH experienced by the sample inside. The oven setup involves targeting a % RH outside, but factors like temperature, oven pressure, and dimensions affect the % RH experienced by the sample inside. Increasing temperature increases the kinetic energy of molecules, leading to higher vapour pressure and more water molecules transitioning into the vapour phase, causing a decrease in % RH with higher temperatures. The final version of the drying

model includes calculations for % RH inside the oven using the Antoine equation, vapour pressure values, and an updated calculation for the wet bulb temperature (T_w) based on the % RH value inside the oven and a specific equation for an air-water system. The model enables predictions of % Moisture Content versus Time. Experiments were conducted to compare the model's predictions with experimental data during the constant rate period. The model's predictions slightly either underestimated or overestimated drying, with differences less than 5% in each experiment. The DVS data used to inform the model is highly accurate, repeatable, and derived from a well-controlled environment with stable parameters. However, the oven used in the experiments has less controllable parameters, leading to fluctuations in temperature, % RH, and pressure. These fluctuations add complexity to the prediction of drying times in the oven compared to the more controlled DVS setup. The development work done to date on the model, incorporates all parameters and properties that have been raised or found to be important key factors in predicting drying times for an air-water system under convective drying.

7.2. Main findings

- Hydrate stability for both model compounds was only found at 75% RH and 25°C, with the stability lasting the entire 12-week experimental period. Under all other conditions samples transformed back to their original starting materials.
- During the previously mentioned stability study, for both compounds, changes in PSD did not positively impact or lead to the preservation of either hydrate form.

- The DVS Intrinsic proved to be a very useful instrument for the collection of comprehensive drying data from wet cakes for a single solvent system (water). This data enables highly repeatable drying curves to be generated and drying kinetics data to be calculated.
- By plotting Moisture Content vs. Time graphs from the DVS data, a point of inflection was found, and this was confirmed to be the time point between the loosely bound moisture being removed, and the water of hydration being negatively impacted and lost.
- Through a small-scale drying study using the DVS, it was found:
 - For both hydrate forms, drying times increased with an increase in % RH and decreased with an increase in temperature. The calculated drying rate decreased with an increase in %RH.
 - Stability of CBZ dihydrate was found in environments $\geq 40\%$ RH (40°C). Further work found that at 40% RH (40°C) stability was lost after ~150 min, whereas at 60% RH (40°C), stability was seen throughout a 12 hr hold time.
 - Effects of PSD on drying times for CBZ dihydrate saw Large crystals dried the fastest, which was an unexpected finding, but that dehydration (loss of water of hydration) took the longest for Large crystals. Small and Medium had similar drying times.
 - Increasing the sample size (increasing cake depth) for CBZ dihydrate caused a doubling in the drying time.
 - Stability of THEO monohydrate was evident in environments $\geq 40\%$ RH. Examining closer, it was found that at 40% RH (40°C), stability was lost after ~300min, whereas at 60% RH (40°C), stability was seen throughout a 12 hr hold time. In comparison to CBZ dihydrate, THEO

monohydrate showed greater stability at 40% RH (40°C), and this is likely due to strong interactions within the monohydrate structure.

- The effect of PSD on drying showed that drying time increased with PSD for the monohydrate.
- Increasing the sample size (increasing cake depth) resulted in only a 3 min increase in drying time.
- During model development, predicting X_C and h_C directly from the experimental DVS data was found to be more accurate than previous model versions, where h_C was calculated using the Nusselt number and an assumption for X_C was made based on drying rate curves from the DVS data.
- Using the Antoine equation proved crucial and helped to relate the % RH set on the outside of the oven to what was being experienced inside the oven.
- By having a value for the % RH inside the oven (previous point), this meant an updated T_w calculation for air-water systems could be used. Experimental data found that the prediction using these updates, compared very well to experimental data gathered from within the constant rate period.

7.3. Future work

- Regarding the model and the series of calculations being used to predict drying times, another equation has been suggested, the Clausius Clapeyron equation. The main aim of the Clausius-Clapeyron equation is to describe the relationship between the vapor pressure of a substance and its temperature. It provides a quantitative understanding of how the vapor pressure of a substance changes with temperature. It establishes a direct

relationship between these two variables. The equation is often written in the form:

$$\ln \frac{P_2}{P_1} = \left(\frac{\Delta H_{vap}}{R} \right) * \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad \text{Eq. 38}$$

Where P_1 and P_2 are the vapor pressures at temperatures T_1 and T_2 respectively, ΔH_{vap} represents the molar enthalpy of vaporization, R is the ideal gas constant, and $\ln$ denotes the natural logarithm.

- The equation demonstrates that as the temperature increases, the vapor pressure of a substance tends to rise. It also shows that the slope of the relationship is influenced by the enthalpy of vaporization. The Clausius-Clapeyron equation is particularly useful for estimating vapor pressure at different temperatures, determining boiling points, studying phase diagrams, and understanding the behaviour of substances undergoing phase transitions. Using this equation could provide a more simplistic approach to the calculations.
- The use of a case study compound, a 'known' unknown, to further validate the model: A third channel hydrate, such as brucine, ^[21] whose hydrate formation is made in an aqueous environment, similar to CBZ dihydrate and THEO monohydrate, should be investigated. Brucine has three hydrated forms, both stoichiometric and non-stoichiometric, providing more
- Investigations should also be made into the ability of the model to predict drying times for different types of hydrates (isolated-site, dapsone, or ion coordinated hydrates, ondansetron hydrochloride). ^[21]
- Expanding the modelling work to enable use with other lab scale dryers like the AFD. Calculations would need to include agitation which would affect heat transfer coefficients of the current model. AFD is a commonly used type of dryer within

the pharmaceutical industry so making the model inclusive of this could be of great advantage.

- Using DVS to investigate organic and binary systems would then be the next important step. The work in this project looked at aqueous systems only and the DVS system used for the drying study only had aqueous capabilities. Based on the data gathered during this project and its potential, a new DVS instrument has been purchased by the Enterprise Partner (APC Ltd.). This new DVS has the additional capabilities of a larger temperature range, aqueous and organic reservoirs for the analysis, either singly or in combination, and it also has the option of modular capabilities like Raman, microscopy, and video. By carrying out similar drying studies, varying temperature and % RH, partial pressure of the organic solvent, and PSD, it would allow for a library of drying kinetics data to be collected for various compounds.
- Regarding the scale-up work within the dryers for the validation of models the incorporation of PAT such as Raman and NIR could provide more precise end point of drying data, allowing for the model to be built upon and get closer to the true end point for any specific sample. The inclusion of probes for the direct measurement of wet bulb temperatures and % RH levels inside the lab dryers would allow for fewer assumptions to be made via the model itself.
- Ultimately, the end goal of this work is to know the minimum amount of DVS experiments and other analyses (SEM, PSD, microscopy) required to provide enough information to feed into the model for it to be able to predict the drying time of a hydrate or solvate. Being able to achieve compound specific drying time predictions through a minimal number of DVS experiments and using small amounts of sample is advantageous.

- Future work on a much larger scale would involve the use of this model in a manufacturing-scale process. Based on ICH Q8 for pharmaceutical development, to do this, significant scale-up work would be required to justify the relevance of a design space developed at small (DVS) and lab-scale (convective oven drying). This would involve work on a larger lab scale, then to pilot scale and finally to the proposed production scale manufacturing process. Systematic scale-up allows time for understanding how one scale relates to the other, any additional parameters that come into play at larger scale (e.g., dryer design configurations, condition changes due to scale change, and heat transfer resistances) and the potential risks that could be encountered in the scale-up operation.

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Appendix A. PSD Data

Appendix A1. PSD data of Small CBZ dihydrate crystals.

PR041				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	A580_PR041 - Small - 1	2.86	12.00	125.00
	A580_PR041 - Small - 1	2.81	11.50	114.00
	A580_PR041 - Small - 1	2.77	11.20	115.00
	A580_PR041 - Small - 2	2.95	14.30	134.00
	A580_PR041 - Small - 2	2.89	13.10	110.00
	A580_PR041 - Small - 2	2.85	12.40	99.50

Mean		2.86	12.42	116.25
Std Dev		0.06	1.04	10.94
RSD (%)		2.05	8.39	9.41

Appendix A2. PSD data of Medium CBZ dihydrate crystals.

PR063				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	A580_PR063 - Medium - 1	2.57	12.60	423.00
	A580_PR063 - Medium - 1	2.53	11.80	362.00
	A580_PR063 - Medium - 1	2.54	12.00	386.00
	A580_PR063 - Medium - 2	2.59	12.80	299.00
	A580_PR063 - Medium - 2	2.49	11.20	232.00
	A580_PR063 - Medium - 2	2.45	10.60	207.00
Mean		2.53	11.83	318.17
Std Dev		0.05	0.76	79.23
RSD (%)		1.86	6.43	24.90

Appendix A3. PSD data of Large CBZ dihydrate crystals.

PR067				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	A580_PR067 - Large - 1	29.40	585.00	1150.00
	A580_PR067 - Large - 1	36.20	650.00	1290.00
	A580_PR067 - Large - 1	33.10	587.00	1100.00
	A580_PR067 - Large - 2	36.20	632.00	1210.00
	A580_PR067 - Large - 2	33.60	589.00	1100.00
	A580_PR067 - Large - 2	32.40	553.00	1020.00
Mean		33.48	599.33	1145.00
Std Dev		2.34	32.26	86.55
RSD (%)		6.98	5.38	7.56

Appendix A4. PSD data of Small THEO monohydrate crystals.

PR061				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	A580_PR061 - Small - 1	11.50	35.90	171.00
	A580_PR061 - Small - 1	11.30	34.40	149.00
	A580_PR061 - Small - 1	11.20	33.50	135.00

	A580_PR061 - Small - 2	13.10	39.40	157.00
	A580_PR061 - Small - 2	13.00	38.80	149.00
	A580_PR061 - Small - 2	13.00	39.30	159.00
Mean		12.20	36.90	153.00
Std Dev		0.93	2.63	12.20
RSD (%)		7.63	7.15	7.96

Appendix A5. PSD data of Medium THEO monohydrate crystals.

PR058				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	A580_PR058 - Medium - 1	13.80	47.50	206.00
	A580_PR058 - Medium - 1	13.80	47.60	208.00
	A580_PR058 - Medium - 1	13.70	47.10	200.00
	A580_PR058 - Medium - 2	13.80	47.30	188.00
	A580_PR058 - Medium - 2	13.80	47.20	189.00
	A580_PR058 - Medium - 2	13.80	46.80	184.00
Mean		13.80	47.20	196.00
Std Dev		0.06	0.29	10.30
RSD (%)		0.41	0.60	5.27

Appendix A6. PSD data of Large THEO monohydrate crystals.

PR062				
	Sample Name	D10 (µm)	D50 (µm)	D90 (µm)
	INVA580_1-157-PR062_1	20.00	66.50	199.00
	INVA580_1-157-PR062_1	19.50	64.70	193.00
	INVA580_1-157-PR062_1	19.10	63.00	186.00
	INVA580_1-157-PR062_2	22.80	76.10	218.00
	INVA580_1-157-PR062_2	22.30	74.50	216.00
	INVA580_1-157-PR062_2	21.80	72.60	209.00
Mean		20.90	69.60	204.00
Std Dev		1.59	5.51	12.80
RSD (%)		7.59	7.91	6.27