

# **Assessing Process Effects on the Recrystallisation of Co-Amorphous Form Products**



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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. Where others have contributed this has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The candidate was responsible for carrying out the experiment work and data analysis. The manuscript was written by S. Roy with a supervisory contribution from A. Brown, E. Martin, and F. Costa.

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## Abstract

Amorphous drug delivery systems can improve the performance of poorly soluble, active pharmaceutical ingredients (APIs) especially for category II and IV APIs of the Biopharmaceutics Class System. However, poor physical stability of an amorphous form can hamper application to drug delivery. To overcome this challenge, co-amorphous systems (CASs) can be obtained by combining an API with a small excipient molecule. In this work, different co-amorphisation methods were applied to valsartan (VAL), an angiotensin II receptor blocker with poor aqueous solubility (Class II of BCS), and nicotinamide (NIC), an effective hydrotrope to enhance solubility of the VAL. Multiple CASs of varying ratios of VAL to NIC were prepared by melt amorphisation and characterised by X-ray, microscopic, thermal and, spectroscopic analyses. The CASs were X-ray amorphous, dominated by a single phase with multiple VAL-NIC binding sites and show enhanced aqueous solubility of VAL, but hydration of the NIC was shown to lower the glass transition temperature below that of the nominal starting composition, particularly for the NIC-rich CASs. The equimolar system was identified as optimal because it retained the least crystallinity with the best aqueous solubility of VAL and was the most stable against recrystallisation. CASs of equimolar amounts of VAL and NIC were then prepared by different processes to produce X-ray amorphous systems, all of which demonstrated enhanced aqueous solubility and dissolution rates. Inhomogeneous mixing during the different preparation routes was identified by polarised light microscopy, which showed a range of retained crystallinity in all samples. Systems prepared by solvent evaporation techniques demonstrated the best VAL-NIC mixing, aqueous solubility, and dissolution rate, suggesting these approaches are ideal for making a homogenous, single-phase CAS. The as-produced, equimolar CASs were subjected to ageing in different relative humidities, with characterisation every two weeks to identify the early stages of recrystallisation. Slurry mixing was the most effective against recrystallisation in 85% RH, still retaining the CAS's X-ray amorphous state at 32 weeks and having 20 times better solubility than VAL alone. The main conclusion of this study is that the stability and solubility of each CAS was limited by amorphous phase separation and not recrystallisation (although both are enhanced in the presence of moisture). The next steps would be to understand reasons for inhomogeneous mixing during processing and the resulting effect on phase separation and recrystallisation in CASs will lead to improvement of these drug delivery systems.

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## Abbreviations

|              |                                         |
|--------------|-----------------------------------------|
| <b>AAPS</b>  | Amorphous-Amorphous Phase Separation    |
| <b>ACE</b>   | Angiotensin-Converting Enzyme           |
| <b>Am</b>    | Amorphous                               |
| <b>API</b>   | Active Pharmaceutical Ingredient        |
| <b>ARB</b>   | Angiotensin (II) Receptor Blocker       |
| <b>ASD</b>   | Amorphous Solid Dispersion              |
| <b>BCS</b>   | Biopharmaceutical Classical System      |
| <b>BM</b>    | Ball Milled                             |
| <b>CAS</b>   | Co-Amorphous System                     |
| <b>DFT</b>   | Density Functional Theory               |
| <b>DSC</b>   | Differential Scanning Calorimetry       |
| <b>FT-IR</b> | Fourier Transform Infrared Spectroscopy |
| <b>GFE</b>   | Gibbs Free Energy change                |
| <b>GT</b>    | Gordon Taylor                           |
| <b>HPLC</b>  | High-Performance Liquid Chromatography  |
| <b>IE</b>    | Interfacial Energy                      |
| <b>MA</b>    | Melt Amorphised                         |
| <b>NIC</b>   | Nicotinamide                            |
| <b>PDF</b>   | Pair Distribution Function              |
| <b>PLM</b>   | Polarised Light Microscopy              |
| <b>pXRD</b>  | Powder X-Ray Diffraction                |
| <b>RH</b>    | Relative Humidity                       |
| <b>SC</b>    | Semi-Crystalline                        |

|              |                                                         |
|--------------|---------------------------------------------------------|
| <b>SD</b>    | Spray Dried                                             |
| <b>SEM</b>   | Scanning Electron Microscopy                            |
| <b>SL</b>    | Slurry Mixed                                            |
| <b>TA</b>    | Thermal Analysis                                        |
| <b>TGA</b>   | Thermogravimetric Analysis                              |
| <b>tmDSC</b> | Temperature Modulated Differential Scanning Calorimetry |
| <b>VAL</b>   | Valsartan                                               |
| <b>VFE</b>   | Volume-Free Energy                                      |

## Chapter 1. Introduction

### 1.1. Background

Active pharmaceutical ingredients (APIs) are broadly divided into four categories based on their solubility and permeability by the United States Food and Drug Administration's Biopharmaceutical Classification System (BCS) (1). Around 90% of drugs in development and 40% of drugs on the market have poor aqueous solubility (2, 3). Inconsistent bioavailability and poor solubility of drugs are the main physiochemical properties that pose a challenge in the formulation of medications, especially in the cases of drugs belonging to the BCS Class II category, which are defined as containing APIs with low solubility and high permeability (4, 5). There have been multiple ways of preparing drug delivery systems that aid in the improved solubility of these APIs. Amorphous solid dispersions (ASDs) tackle the problem of poor solubility, where the APIs are amorphised and are combined with polymers to enhance solubility and stabilise the systems. However, the higher pill burden of these formulations along with hygroscopicity of the polymers which can facilitate recrystallisation, makes ASDs an unfavourable option. Co-amorphisation can overcome the challenges of recrystallisation and low solubility of APIs. There are a couple of ways of developing co-amorphous systems (CASs). It can be by combining an API with a low molecular weight co-former (drug-excipient) or another drug (drug-drug), to form a single-phase homogenous amorphous system (6-12). These systems possess intermolecular interactions that enhances miscibility, thereby promoting higher levels of molecular mixing (12). The solubility of the poorly soluble API increases in a CAS when the excipient has high solubility as the higher solubility and dissolution of the co-former aids in the release of the API from the CAS, thus aiding in more efficient absorption of the drug (13-16).

Cardiovascular diseases are the global leading cause of death, with an estimated 17.9 million deaths in 2019 and this has risen to 20.5 million deaths in 2025 and is projected to go up to 35.6 million fatalities in 2050, making cardiovascular research very significant for the betterment of health and well-being (17, 18). Valsartan (VAL), a BCS class II drug, is primarily delivered orally in the form of tablets and capsules, and it tackles heart failure, diabetic nephropathy hypertension, reducing the risk of both fatal and non-fatal cardiovascular events such as myocardial infarction (19). It is often used on its own, or in combination with another active substances for patients with heart failure (20, 21). The global popularity and market for VAL is high and is expected to reach 2.51 billion

USD by 2035, with a compound annual growth rate of 5.11% from 2025 to 2035 (the United States of America dominating the market), making it a very popular antihypertensive medication (22, 23). It binds to and inhibits the hypertensive activity of Angiotensin II receptor type 1 (AT<sub>1</sub>) by relaxing and widening blood vessels, thus lowering blood pressure. Persistent hypertension is a leading cause of cardiovascular diseases, especially heart attacks, strokes, heart failure and aneurysms (24). VAL is a poorly (water) soluble API, which makes its absorption limited by its dissolution rate as the body cannot absorb the API faster than it dissolves, thus rendering it inefficient (25). Hence, it is important to develop a delivery system that enhances the solubility and stability of VAL. Low molecular-weight excipients are small molecules that can bind to drugs during development of a co-amorphous drug delivery system. Optimum co-former selection is imperative as they often do not produce fully amorphous CASs (26-28). For this research, nicotinamide (NIC) has been taken as the excipient for the co-amorphous formulations, based on the work of Turek *et al.*, who show it to be an effective co-former (10). NIC is a water-soluble form of vitamin B<sub>3</sub> (hydrotrope) with high bioavailability, that is naturally occurring in different kinds of food, making it an excellent choice for CAS formation (29-31).

## 1.2. Gap in Research

Although CASs improve solubility and dissolution rates compared to a lone API, the long-term stability of the systems have generally only been investigated under little to no humidity at low temperature, where CASs' are able maintain an amorphous state for up to two years (10, 26, 28, 32-40). Very few studies have been conducted on co-amorphous systems where the systems are stored at relatively moderate to high humidity at elevated temperatures (6, 27, 41-43). For a co-amorphous system to destabilise and undergo recrystallisation, it would have to transform from a single homogeneous phase to heterogeneous phases, by overcoming the secondary bonding drug-excipient interactions that are holding the primary phase together, undergo clustering to form stable nuclei and ultimately (re-)crystallise. Such a phase transformation could occur differently in different formulations, so each need to be investigated. In addition, although an excipient hydrotrope such as NIC may aid in the solubilisation of an API in a CAS, its polar nature allows it to form strong interactions with water molecules, which can plasticise the amorphous phase (increasing molecular mobility) or even be strong enough to overcome the interaction between NIC and the API molecules, leading to the disruption

of the bonding association within the drug system, thus potentially facilitating phase separation and recrystallisation of NIC (44). Although Turek *et al.* had successfully prepared co-amorphous systems of VAL and NIC by different methods such as mechanical mixing (ball milling) and solvent evaporation (slurry mixing), the stability of these systems was not explored beyond storage under dry conditions, where the effect of moisture on the CASs was not investigated, nor did they explore the effect of varying the molar ratios of VAL and NIC CASs on solubility and crystallisation. Different routes of preparation for systems with the same components can themselves produce different products, in terms of particle sizes, phases or the overall homogeneity of the systems, which may impact their performance, making it vital to investigate how different modes of preparation affect the performance of systems having the same key components (10, 45, 46).

Long-term stability studies on co-amorphous formulations are necessary to be conducted at different relative humidities to identify stability against phase separation, under time, moisture, or both, and eventually against recrystallisation, because this can adversely affect the performance of drug delivery systems in terms of solubility and dissolution rates. Co-amorphous systems are often stabilised by hydrogen bonding interactions and have enhanced aqueous solubility prior to any ageing studies, but it is important to understand how these systems would perform in high humidity and whether they maintain long-term stability in these conditions. Additionally, the recrystallisation pathways of binary systems have not been fully explored and it would be interesting to study how components can undergo nucleation in these systems and which preparation technique can be used to produce a co-amorphous system that gives the best performance against recrystallisation. There is also a possibility of a co-amorphous drug delivery system becoming hydrated when stored at high humidities due to adsorption of water by any components, and this may affect molecular mobility and physical stability, making it necessary to investigate the behaviour of co-amorphous form products stored under high humidity (47).

This project will assess the effects of different processing techniques on the production of VAL and NIC CASs and will check for amorphous phase separation over time along with measuring stability against recrystallisation under moisture.

## Chapter 2. Literature Review

### 2.1. Chapter Overview

This chapter will briefly explore pharmaceutical research including the achievements and the shortcomings in its development, especially in terms of the development of amorphous form products. Amorphous drug delivery systems can improve the performance of poorly soluble active pharmaceutical ingredients (APIs) especially for Class II and IV APIs of the Biopharmaceutics Class System (48). However, poor physical stability of the amorphous forms hampers the application in drug delivery. To overcome this challenge, co-amorphous systems (CAS) can be obtained by combining an API with a small molecule. In this work, different co-amorphisation methods were applied to valsartan (VAL), an angiotensin II receptor blocker with poor aqueous solubility (BCS Class II), and nicotinamide (NIC), a hydrotrope with excellent solubilising properties (10, 31). The limitations associated with analysing pharmaceutical compounds using bulk methods will be presented along with advantages of localised analytical technique. Research on cardiovascular drugs will be showcased in this chapter, leading up to the use of VAL and NIC in the preparation of co-amorphous system in this project, thereby building up the foundation of potential co-amorphous formulation for cardiovascular drugs. This work will also identify how long-term storage under extreme humidity affects the specific model system introduced in this thesis. All the figures presented in this chapter have been prepared in Microsoft PowerPoint.

### 2.2. A brief history of medicinal research

Medicine is the field of science that deals with healing and caring for patients by mitigating injury or disease and supporting their health and well-being. This has been practised since prehistoric times, with the earliest record of dental treatment discovered in a burial in Belluno (Italy) within a member of the Late Upper Palaeolithic society (circa 12000 BCE). This finding has been supported by multiple modern analytical techniques such as scanning electron microscopy and mass spectroscopy analysis (49). The growth in the understanding of the human physiology was taking place across civilisation through the ancient world.

Hippocrates of Kos, a Greek Physician, hailed as the “Father of Medicine” established the school of medicine, separate from religion and superstition. His work in disease classification and diagnosis based on clinical observation is the foundation of

modern-day medicine and his work towards medical ethics such as the Hippocratic Oath is still in use today (50, 51). Erasistratus of Ceos, a Greek royal physician laid down the foundation of human physiology by studying the nervous system and the heart (52). Syrian polymath Ibn al-Nafis discovered pulmonary and coronary circulation and this knowledge has been developed further by Realdo Colombo of Italy and their work formed the foundation of the circulatory system by William Harvey of England (53-55). Development of the modern-day medicine can be traced back to the conception of Germ theory established and expanded by the French chemist Louis Pasteur and German bacteriologist Robert Koch (56, 57). However, the foundation of this theory can be traced back to the recorded works of Marcus Terentius Varro and by Girolamo Fracastoro's Theory of Contagion as well as the clinical observations of physician Louis-Daniel Beaupérthuy, Florence Nightingale of England, Ignaz Semmelweis, Carl Mayrhofer, John Snow and Joseph Lister (58-63). This theory is the foundation to modern drug development and laid the groundwork for the development of antibiotics and vaccines.

### 2.3. Development of drug delivery systems

Active pharmaceutical ingredients (APIs) are the drug substances which when combined with other components, namely amino acids, lipids, polymers, or other drugs can treat diseases. Such drug substances are broadly divided by the United States Food and Drug Administration's Biopharmaceutical Classification System (BCS) into four categories based on their solubility and permeability, as tabulated in [Table 2.1](#), and developed from the work of Amidon *et al.* 1995 (1). Approximately 90% of drugs in development and 40% of drugs on the market have poor aqueous solubility (2, 3). Inconsistent bioavailability and poor solubility of drugs are the main physiochemical properties that pose a challenge to the formulation of medications, especially in the cases of drugs belonging to the BCS Classes II (drugs with high permeability and low solubility) and IV (drugs with low permeability and low solubility) (4, 5). Solubility is a significant factor in the efficacy of a drug, thus poor solubility is a constraint in the formulation of effective medicines (64). Furthermore, the dissolution rate of a drug system is dependent on the solubility of the drug system, and has an impact on the bioavailability of a drug (65). Drugs with poor solubility lack the mobility to move into the blood stream, resulting in low bioavailability and low efficiency (66-68). The low solubility of APIs can be attributed, in part, to their crystalline structure, which possesses low energy and mobility (68).

**Table 2.1:** Biopharmaceutical classification system (BCS) where APIs are classified in four categories in accordance with their capability to permeate through biological membrane and aqueous solubility.

| <u>CLASS</u>                 | <u>SOLUBILITY AND PERMEABILITY</u>                                                               | <u>EXAMPLES</u>                                                                         |
|------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>BCS Class I (69)</b>      | <ul style="list-style-type: none"> <li>• High Solubility</li> <li>• High Permeability</li> </ul> | <ul style="list-style-type: none"> <li>• Paracetamol</li> <li>• Theophylline</li> </ul> |
| <b>BCS Class II (43, 70)</b> | <ul style="list-style-type: none"> <li>• Low Solubility</li> <li>• High Permeability</li> </ul>  | <ul style="list-style-type: none"> <li>• Nateglinide</li> <li>• Ibuprofen</li> </ul>    |
| <b>BCS Class III (70)</b>    | <ul style="list-style-type: none"> <li>• High Solubility</li> <li>• Low Permeability</li> </ul>  | <ul style="list-style-type: none"> <li>• Cimetidine</li> <li>• Amoxicillin</li> </ul>   |
| <b>BCS Class IV (71)</b>     | <ul style="list-style-type: none"> <li>• Low Solubility</li> <li>• Low Permeability</li> </ul>   | <ul style="list-style-type: none"> <li>• Ritonavir</li> <li>• Furosemide</li> </ul>     |

To tackle this problem, poorly soluble crystalline drug forms of the BCS Classes II and IV can be transformed to amorphous counterparts which lack long-range order and periodicity (67, 68). Furthermore, amorphous forms of APIs have higher free energy, the molecules are randomly oriented and possess large surface area due to reduced particle size, all of which enhances wettability by increasing the exposure of functional groups (67). This can increase solubility and raise saturation concentration in solution which would increase dissolution rate (68, 72). Such an increase would enhance the bioavailability of an API with respect to its crystalline form.

Amorphous forms are thermodynamically unstable and are prone to recrystallisation. Structural instability of amorphous APIs escalates when subjected to moisture because water behaves as a plasticiser to lower glass transition temperatures ( $T_g$ ), thereby increasing molecular mobility and promoting recrystallisation (73). Hence, it is imperative to find a way of stabilising amorphous forms by introducing a secondary stability component to the API, i.e., transforming the formulation into a drug delivery system (DDS). Drug delivery systems (DDSs) are a process of administering a specific amount of pharmaceutical ingredient to the desired target site within the body to achieve the desired therapeutic effect whilst sustaining the required drug intensities (74). A secondary component to a DDS can be a polymer, an amino acid, a lipid or even another drug (8, 10, 75, 76).

Drug delivery systems can broadly be classified into two categories based on either their mode of administration or their formulation as demonstrated in [Figure 2.1](#).

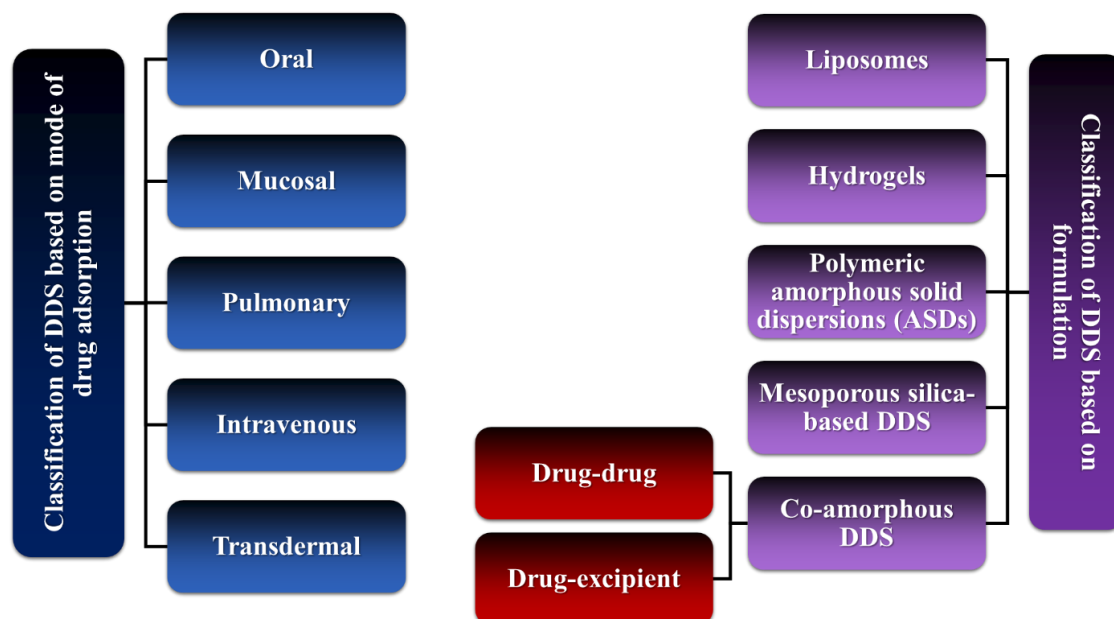


**Figure 2.1:** Different drug delivery systems (DDSs) based on administration and formulation.

The drugs that are classified under routes of drug adsorption/administration can be further divided in five categories, namely oral or ingestion, mucosal, pulmonary or inhalation, intravenous or injection and transdermal (74, 77). Oral DDSs are the most widely used modes for systemic and gastrointestinal maladies (78). Oral DDS are the drug systems where the dosage is administered by mouth in the form of a pill or tablet or syrup for localised therapeutic action or systemic absorption within the gastrointestinal tract (stomach or the intestine). Oral DDSs are the most popular form of DDSs and drugs such as valsartan (hypertension), glipizide (antidiabetic), ritonavir (antiretroviral), and many such APIs are combined with a secondary compound to deliver an intended therapeutic effect (6, 10, 79). Pulmonary DDSs are a route of administration of the drug directly to the lungs by means of inhaler or nebuliser facilitating speedy systemic absorption and release of medication (80). Salbutamol, on its own or in combination with polymers has been implemented to treat fibrosis, i.e., as a pulmonary treatment (81, 82). Transdermal DDSs are a non-invasive, controlled approach of administering a drug where an adhesive patch is applied to the skin, such that a specific dose of medication is absorbed through the skin into the bloodstream, thereby bypassing the acidic conditions of the gastrointestinal system (83). Nicotine replacement therapy is an example of a transdermal DDS that delivers regular doses of nicotine through the dermis to combat withdrawal

symptoms associated with nicotine addiction (84). Intravenous DDSs are drugs that are directly injected into the bloodstream, and have a more immediate effect with up to 100% bioavailability (85). Etomidate is a popular intravenous agent with excellent hemodynamic stability, either in solution or as a lipid emulsion, and is used as a DDS for anaesthesia (86, 87). Mucosal DDSs are non-invasive drug formulations (gel, patches, tablets) where the API delivers its therapeutic influence by the body's moist protective membranes such as oral, ocular, nasal, vaginal, and rectal pathways, thereby bypassing the gastrointestinal and liver metabolism (88). Astodimer is an example of a topical mucosal DDS in the form of a gel for the treatment of bacterial vaginosis (89).

The secondary category of classification of DDSs is based on their formulation such as polymeric amorphous solid dispersions (ASDs), liposomes, mesoporous silica, hydrogels, and co-amorphous systems. Hydrogel drug delivery systems comprise of an API embedded in three-dimensional polymeric networks i.e., hydrogels. They are biodegradable and hydrophilic, thereby exhibiting high water absorption and retention capabilities. These swollen polymer matrices can mimic natural tissues which along with their biocompatibility and resistance against enzymatic degradation, enables them to repair damaged gastrointestinal tissues (90, 91). The permeable assembly of the polymeric networks in hydrogels are excellent for drug encapsulation, assists in controlled release of APIs in the gastrointestinal tract and protects it from rapid metabolic degradation. However, this system is not compatible with hydrophobic components, making it incompatible with BCS Class II and IV drugs.

A liposome is a small artificial vesicle, spherical in shape, having at least one lipid bilayer and **is capable of encapsulating** water-soluble and fat-soluble substances. It is often composed of phospholipid molecules of soy, egg, milk, and even piscine phospholipids, that assemble on their own in aqueous solution, existing as bilayers that mimic cell membranes (90, 91). These complex structures can be combined with BCS Class II and IV drugs as they are comprised of both hydrophilic and hydrophobic components. Although they are a favourable match for poorly soluble drugs, they have poor drug loading efficiencies and DDSs based on these are often left with residual solvents. Furthermore, these systems are prone to forming aggregates which can potentially build up excess pressure within the DDS leading to disruption, thereby causing uncontrolled release of drugs, which defeats the efficacy of the DDSs (90-92).

In mesoporous silica-based drug delivery systems, the drugs are mixed with silica or silicon dioxide (SiO<sub>2</sub>) nanoparticles, which are mesoporous, possessing pores with large total volumes and diameter ranges between 2 to 50 nm, that enable the adsorption of APIs on/in the silica particles, thus forming an amorphous silica matrix, stabilised by nanoconfinement (11, 93). This prevents recrystallisation of the drug as such an occurrence is physically inhibited due the dimension of a pore being smaller than that of the crystal nucleus required to facilitate recrystallisation (94). The disadvantages of using silica-based DDSs lies with their mode of preparation which relies on organic solvents and limited loading capacity, typically up to 500 mg of drug to 1 g of silica (11, 95).

A system with an API and a polymer is known as an amorphous solid dispersion (ASD) (75, 96-98). ASDs enhance solubility and stability of amorphous APIs, but they also reduce molecular mobility of the drug by the very act of introducing intermolecular interactions between the API and a polymer, as is observed in ASDs of felodipine (API)-Soluplus (polymer) and felodipine-HPMCAS (99, 100). In addition, the hygroscopic nature of polymers can have a detrimental effect on the stability of ASDs as the systems may absorb more moisture than an amorphous API alone (101, 102). Such sensitivity to moisture also has a negative influence over the miscibility between an API and a polymer matrix (11, 96, 102, 103). Furthermore, to ensure molecular mixing, high amounts of polymer are required, which leads to high dosage volumes as the polymer matrix often have higher molecular weight compared to the APIs. This affects the formulation of tablets, leading to a high pill burden for patients (104, 105).

Co-amorphisation can bypass the challenges of recrystallisation and low solubility of APIs. There are a couple of ways of formulating co-amorphous systems (CASs). An API can be combined with a low molecular weight co-former, such as an amino acid, amide, carboxylic acids (drug-excipient), or another drug (drug-drug), to form a single phase homogenous amorphous system (6-12, 68). Such systems possess intermolecular interactions that enhances miscibility, thereby promoting higher levels of molecular mixing (12). The small molecules possess high conformational flexibility, making them harder to recrystallise as they are present in a low-energy conformation that would require high activation energy to transition (106, 107). This increases the stabilities against phase separation and recrystallisation (12). Additionally, replacing polymers of higher molecular weight with low molecular weight excipients has a positive effect on the size/scale of the systems as it lessens the quantity of excipient required for the binary system, lowering pill burden, and potentially also reducing the predicament of

hygroscopicity that comes with polymers (11). This makes for higher API concentrations compared to polymer-based solid dispersions. CASs enhance drug solubility as the high energy metastable amorphous phase makes way for faster dissolution and increased solubility relative to a crystalline counterpart. Due to the strong intermolecular interaction between the API and the co-former, they exhibit high stability within the homogeneous mixture, as well as improving dissolution of the API over both the crystalline and individual amorphous forms (14, 40). This enhanced solubility of the CASs can be attributed to the hydrotropic effect of the excipient. Hydrotropy is a phenomenon of solubilisation where the addition of a second component enhances the aqueous solubility of a primary component, as the hydrophobic end of the hydrotrope interacts with the sparingly soluble API whilst its hydrophilic end interacts with water molecules and disrupts the water network to solubilise the compounds (13, 108). CASs can be prepared by techniques such as ball milling, hot melt extrusion and spray drying, which are described in section 2.5.3 and further elaborated in Chapter 3.

## 2.4. Research on cardiovascular diseases

Currently, the research on pharmaceutical development has come a long way since the discovery of the circulatory system, and the field of medical research is ever-expanding. One such field is the research of cardiovascular diseases as these are the global leading cause of death, with an estimated 17.9 million deaths in 2019, that has risen to 20.5 million deaths in 2025 and is projected to go up to 35.6 million fatalities in 2050 (17, 18). Further research on cardiovascular systems could lead to a breakthrough that can help tackle this growing predicament.

The cardiovascular system is made up of the heart and blood vessels, namely arteries, veins and capillaries, supplying oxygen and nutrients to the whole body and removing carbon dioxide and other byproducts through the blood. Cardiovascular diseases are a group of diseases affecting the heart and/or the blood vessels. They can broadly be divided into five categories, namely coronary artery disease (affects the blood vessels supplying blood to the heart), rheumatic heart disease (damages to the muscles of the heart and its valves by the streptococcal bacteria), peripheral artery disease (affects the blood vessels supplying blood to the limbs, congenital heart disease (birth defects in the heart) and cerebrovascular disease (affects the blood vessels supplying blood to the brain), as depicted in Figure 2.2 (109). Antiplatelets (medications that prevent blood clots), anticoagulants (blood thinners), statins (agents that reduces low-density

lipoprotein or LDL cholesterol), thrombolytics (clot-busters), diuretics (water pills), beta-blockers (block adrenaline and reduce strain on the heart muscle) and antihypertensives (blood vessel relaxers) are some of the widely used medications to treat cardiovascular diseases (110). Anti-anginal agents such as ranolazine treat chronic chest pains that are often associated with coronary heart disease (111). Benzathine benzylpenicillin is an injectable antibiotic that treats streptococcal infections, making it a valuable API for the treatment of rheumatic heart diseases (112). Antiplatelet agents such as aspirin reduce the chances of cardiovascular events such as strokes in those afflicted with peripheral artery diseases by preventing the formation of blood clots (113). Anti-arrhythmic drugs such as flecainide regulate irregular heartbeats that are commonly associated with coronary heart diseases in children (114). Cerebrovascular diseases are often treated with drugs such as antihypertensives, antiplatelets, statins, and anti-coagulants to regulate blood pressure to prevent strokes or myocardial infarctions (115).

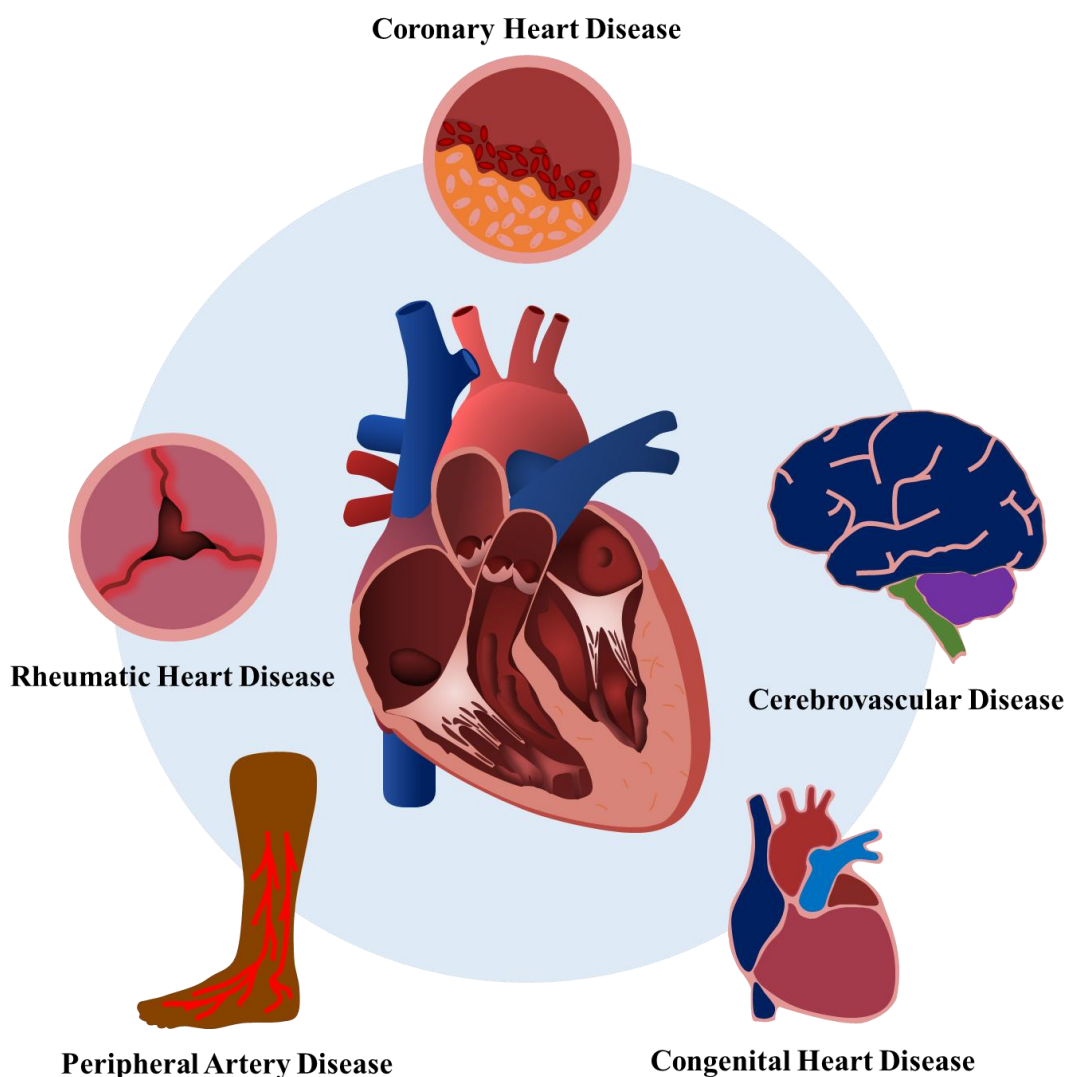


**Figure 2.2:** Cardiovascular Diseases.

Hypertension or high blood pressure (>140/90 mm Hg) when left untreated leads to cardiovascular events. Poor physical health due to a lack of physical activity, old age, poor diet, smoking, and excessive drinking increase the chances of hypertension. Thus, antihypertensive drugs are useful for the treatment of cerebrovascular diseases. Blood relaxers such as Angiotensin-Converting Enzyme or ACE inhibitors, angiotensin II receptor blockers or ARBs, and Calcium-Channel blockers as well as thiazide-based diuretics or a combination of multiple medications are some of the first line of treatments against hypertension (116). Calcium-channel blockers limit the quantity of calcium entering cells of the heart and blood vessels, allowing them to expand and ease the flow of blood. These are commonly prescribed to treat angina along with hypertension (116, 117). Angiotensin-Converting Enzyme or ACE inhibitors relax and widen blood vessels, thus lowering blood pressure and improving the flow of blood, thereby reducing the work done by the heart. Some examples of ACE inhibitor are benazepril, fosinopril, moexipril, captopril, and many more, typically ending with “pril,” and are often used on their own or in combination with diuretics and calcium-channel blockers (118-120). Angiotensin II receptor blockers or ARBs, typically ending with “sartan,” prevent the hormone angiotensin II from binding to AT1 receptors and relax blood vessels. There are six ARBs that are used against hypertension, namely telmisartan, valsartan, losartan, irbesartan, olmesartan and candesartan, and their doses are presented in [Table 2.2](#) (121-123).

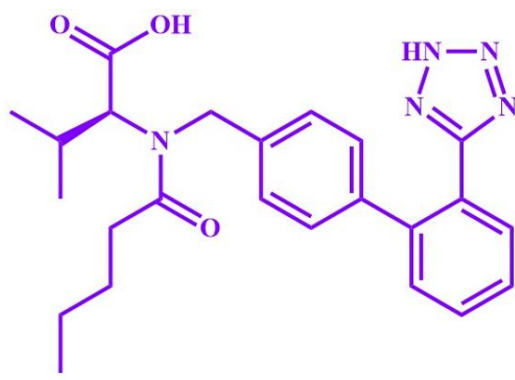
**Table 2.2:** Daily doses of ARBs for hypertension.

| <u>Treatment</u><br><u>ARBs</u> | <u>Low Dose (mg per</u><br><u>day)</u> | <u>Medium Dose (mg</u><br><u>per day)</u> | <u>High Dose (mg per</u><br><u>day)</u> |
|---------------------------------|----------------------------------------|-------------------------------------------|-----------------------------------------|
| Valsartan (124)                 | 80                                     | 160                                       | 320                                     |
| Telmisartan (125)               | 20                                     | 40                                        | 80                                      |
| Olmesartan (126)                | 10                                     | 20                                        | 40                                      |
| Losartan (127)                  | 50                                     | 100                                       | 150                                     |
| Candesartan (128)               | 8                                      | 16                                        | 32                                      |
| Irbesartan                      | 75                                     | 150                                       | 300                                     |

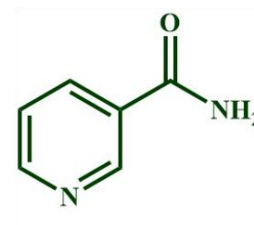
ACE inhibitors and ARBs are administered to patients with chronic kidney disorder and heart failure (116). Although ARBs and ACE inhibitors have similar

effectiveness in terms of the first-line of treatment for hypertension, risks such as pancreatitis, gastrointestinal bleeding, respiratory issues and angioedema (rapid swelling of face or throat) are significantly lower in ARBs than ACE inhibitors, making them the safer and more suitable treatment for hypertension (129).

(S)-3-methyl-2-(N-(38-tetrazolyl-5-yl)biphenyl-4-yl-methyl)pentamido)butanoic acid or valsartan, shown in Figure 2.3, is an antihypertensive drug, sold under the name *diovan*. Valsartan ( $C_{24}H_{29}N_5O_3$  - CAS Reg. No. 137862-53-4), has a molecular weight of  $435.52 \text{ g mol}^{-1}$ , belongs to the type II ARB-II family of drugs which tackle heart failure, diabetic nephropathy hypertension to reduce the risk of both fatal and non-fatal cardiovascular events such as myocardial infarction (19). It can be used by itself or in combination with another antihypertensive component. ARBs relax and widen blood vessels, thereby lowering blood pressure and in addition, valsartan's physiological application leads to a drop in aldosterone levels, tackling cardiac activity. It also plays a role in regulating the sodium level in the body, and its intake increases excretion of sodium, which in turn reduces blood pressure and improves cerebral blood flow by reducing brain amyloid aggregation, and ultimately works towards improving cerebrovascular health (130).



Valsartan (VAL)



Nicotinamide (NIC)

**Figure 2.3:** Chemical structures of valsartan (API) and nicotinamide (excipient).

Persistent hypertension is a leading cause of cardiovascular diseases, especially heart attacks, strokes, heart failure and aneurysms (24). At similar doses, the efficacy of valsartan against hypertension is better in comparison to other ARBs as it is more selective for AT<sub>1</sub> receptors which prevents vasoconstriction, making it a common drug of choice (123, 131). Additionally, valsartan is demonstrated to be safe and tolerable once a day, in a wide variety of patients across age, demographics and physical ailments. It has

been shown to improve blood pressure levels within 2 weeks and maximally by 4 weeks and blood pressure control is maintained for up to 2 years, with no adverse effect on abrupt withdrawal, making it a favourable choice to tackle hypertension (123, 132-134).

Developing better drug delivery systems with APIs that can tackle such a massive health problem is significant. As VAL is a poorly (water) soluble ARB class of API (BCS Class II), it is important to develop a delivery system that has improved solubility and stability of VAL. There are multiple methods of developing drug delivery systems for VAL, which are explored in the next section.

## 2.5. Development of co-amorphous systems

The development of CASs is determined by a few factors, namely secondary component selection, preparation methods, and characterisation techniques that assess the performance of the systems with respect to stability, solubility, and dissolution. A few studies on co-amorphous formulations have been tabulated in [Table 2.4](#).

### 2.5.1. Review of Turek *et al.*

Turek *et al.* developed equimolar CASs of VAL and NIC, as presented in [Figure 2.3](#), by different preparation techniques, namely ball milling, rotary evaporation, and liquid assisted grinding (10). Ethanol was used as the solvent in all three preparation methods. A CAS prepared by slurry mixing (rotary evaporation) was done so by refluxing a solution of the components at 70 °C for three hours before obtaining the powder by solvent removal under vacuum. The CASs prepared by ball milling and liquid assisted grinding utilised a few drops of the solvent during preparation, with the ball milled system being further dissolved in ethanol and subjected to rotary evaporation to obtain a CAS. Liquid assisted grinding was involved in the preparation of two CASs; one had 30 minutes of grinding and the other 60 minutes.

Ball milling and rotary evaporation were successful in making single-phase, equimolar CASs of VAL and NIC, as evidenced by a lack of crystalline peaks in powder (p)XRD patterns and the presence of a single endotherm in a DSC trace that corresponds to the  $T_g$  of the binary system. It is important to note that the CAS prepared by ball milling was combined with rotary evaporation. This combination of ball milling and solvent evaporation may have assisted in the formation of a homogeneous system. Liquid assisted grinding failed to produce any amorphous systems, irrespective of the grinding time, as

evidenced by crystalline peaks in the pXRD patterns and by multiple endothermic events corresponding to multiple  $T_g$ s and a melting event of the pure VAL in the DSC traces.

The bonding interaction between the two components were explored by FT-IR and DFT calculation. FT-IR indicated the formation of hydrogen bonds between VAL and NIC, involving the acid and amide group of VAL and amide group of NIC. The DFT analysis indicates that two heterosynthons can exist in which VAL and NIC are bonded to each other and the experimental data suggest the presence of one heterosynthon is more prevalent than the other. FT-IR data provides information about the polar interaction in systems but fails to account for non-polar interactions. The heterosynthon that is considered to be more predominant shows polar interactions between VAL and NIC and was detected by FT-IR whilst the second heterosynthon, which has non-polar bonding interactions, went undetected. To investigate the secondary interactions between the components, a supplementary characterisation technique must be employed, such as Raman spectroscopy to investigate additional secondary interactions such as changes to C=C bonds (135).

Co-amorphisation enhanced the aqueous solubility of the valsartan prepared by rotary evaporation and ball milling by a factor of 14 and 24, respectively. However, the long-term stability of the systems under high humidity was not well explored. The solvent evaporated CAS and the ball milled CAS maintained an amorphous state for 9 and 11 months respectively by pXRD, with only NIC recrystallising during storage at 0% RH at 25 °C after 9 and 11 months.

To summarise, this body of work proved that co-amorphisation of VAL with NIC enhances the aqueous solubility of VAL, thus providing the opportunity to examine this drug delivery system further. The absence of long-term stability studies of CASs composed of VAL and NIC under high humidity as well as additional studies on the molecular interaction within the binary system and the effect on performance can be investigated further.

### 2.5.2. Secondary Component Selection

Development of CASs is highly influenced by the secondary component in that system, as it strongly influences the performance and stability of the final drug delivery system (105, 136). In the preparation of CASs, the co-former must be generally recognised as safe (GRAS). In a drug-drug combination, both the APIs should be

pharmacologically relevant to one another as well as having an interaction that enhances the stability and performance of a CAS (11, 12, 105, 136). The drug-drug CASs usually tend to focus on the stabilisation of the amorphous form of the API that is being investigated but not enough is known about the biopharmaceutical benefits of this binary system and further study is needed to look at the physiological impact (12).

Low molecular-weight excipients are small molecules that can bind to drugs during the co-amorphous formulation. In the case of a drug-excipient system, amino acids are a favourable option over polymers. Amino acids have lower bulk volume relative to polymers (68). They have higher solubility, whose presence in the CAS can enhance the solubility of the API (11, 14). Amino acids are classified as GRAS and are non-toxic, making them a suitable choice for co-amorphous formulations (28, 68). Co-former selection is imperative as they often do not produce fully amorphous CASs (26-28). For instance, tyrosine was incapable of producing CASs, with either indomethacin or carbamazepine as evidenced by crystalline peaks in the pXRD patterns of the products (28). This is despite tyrosine being able to bind to the same enzyme as indomethacin or carbamazepine (28). Furthermore, intermolecular interactions between the API and the co-former are responsible for the stabilisation of CASs. Hydrogen bonding,  $\pi$ - $\pi$  stacking and van der Waals forces can each contribute towards the formulation of the CASs, and all of which raise the activation energy against recrystallisation. This physical stabilisation of CASs can improve solubility and dissolution rates if the excipient remains highly soluble. By selecting a suitable excipient, not only is the CAS stabilised but it also creates the “Spring and Parachute” effect, where a highly soluble excipient releases an amorphous API rapidly when immersed in a solvent like a compressed spring releasing its energy, maximising API supersaturation in the solvent while it (the excipient) acts like parachute “catching the API”, inhibiting its (API’s) precipitation into a crystalline form (10, 44, 137-141). Hydrogen bonding interactions are stronger compared to the other interactions, thus forming hydrogen-bonded heterosynthons stabilise the CASs and these heterodimers are indicative of the miscibility of the components in the CASs, as evidenced by a singular glass transition events that suggests the formation of single phase homogeneous systems, that are resilient against phase separation and ultimately recrystallisation (10, 137, 142). Furthermore,  $\pi$ - $\pi$  stacking stabilises CASs due to the additional interaction between the aromatic rings of the API and the co-former (137).

For this research, nicotinamide (NIC) has been taken as the excipient for the co-amorphous formulations, based on the work of Turek *et al.* (10, 13, 143). NIC (structure

in [Figure 2.3](#) –  $C_6H_6N_2O$ , CAS Reg. No. 98-92-0) is the chemical 3-pyridinecarboxylic acid amide, with a molecular weight of  $122.12 \text{ g mol}^{-1}$ . It is also known as niacinamide, is a water-soluble amide form of vitamin B<sub>3</sub> (niacin), a nutraceutical with high bioavailability, making it an excellent choice for CAS formation (144). It is a non-micellar hydrotrope that enhances the solubility of poorly soluble APIs, by forming  $\pi$ - $\pi$  interactions with the API molecule (aromatic stacking complexes) whilst interacting with the water molecules by forming hydrogen bonds that disrupts their tetrahedral network (chaotropicity), and forming cavities in the network, making the dissolution of the API easier, leading to enhanced solubility (13, 14, 31, 108, 145-148). NIC is a very popular choice of excipient for the formulation of CASs, as it enhances the solubility and stability of CASs (149). Although NIC is an excellent hydrotrope, moisture sets a limitation on the capabilities of NIC in a CAS as water can act as a plasticiser, which increases the molecular mobility of NIC and lowers the  $T_g$  of NIC and or the binary systems, even where moisture has no plasticising effect on the other component in a CAS (149). As a small, rigid molecule with high molecular mobility and high crystallisation tendency, NIC easily reverts to its crystalline state, which may negate its solubilising abilities as NIC's strong self-aggregation tendency leads to reduced monomer availability for associating with the API, thereby reducing its hydrotropicity, which may pose a challenge (31). Hence, a minimum concentration of NIC is to be required to establish interactions between the NIC and API that are strong enough to facilitate solubilisation but not enough to facilitate self-aggregation (14, 31, 146). However, this project aims to explore this further and determine the influence of NIC as an excipient in VAL-based CAS. NIC is catalogued in the Code of Federal Regulations as a GRAS food additive (21 CFR 184.1535) as well as a GRAS nutrient supplement (21 CFR 582.5535) (150-152). It occurs naturally in the body after consuming both animal and plant-based food (30). It has been reported to have several health benefits, such as supporting the cardiovascular system, acts against inflammation, improves cognitive functioning, and reduces anxiety (153-155). Its anti-inflammatory, anti-ageing and anti-hyperpigmentation properties makes it a useful ingredient in cosmetic formulations (156-158). Turek *et al.*, have successfully prepared equimolar CASs of VAL and NIC with different preparation techniques, and a review of this work is presented in [section 2.5.1](#). Chemometric studies on the FT-IR spectroscopic data were conducted on the stable heterosynthons of VAL and NIC, which helped identify the different hydrogen bonds that stabilised the CASs (10).

**Table 2.3:** pK<sub>a</sub> values of valsartan and nicotinamide, calculated using Advanced Chemistry Development (ACD/Labs) Software (© 1994-2024 ACD/Labs) (10, 159).

|                     |                           |      |                                                                                |
|---------------------|---------------------------|------|--------------------------------------------------------------------------------|
| <b>Valsartan</b>    | pK <sub>a1</sub> (acidic) | 3.9  | $\Delta pK_{a1}^1 = 3.6 - 3.9 = -0.3$<br>$\Delta pK_{a2}^1 = 3.6 - 4.7 = -1.1$ |
|                     | pK <sub>a2</sub> (acidic) | 4.7  |                                                                                |
|                     | pK <sub>a</sub> (basic)   | -0.5 |                                                                                |
| <b>Nicotinamide</b> | pK <sub>a</sub> (acidic)  | 13.4 | $\Delta pK_a^2 = -0.5 - 13.4 = -13.93$                                         |
|                     | pK <sub>a</sub> (basic)   | 3.6  |                                                                                |

One way to see if the two components are suitable for the preparation of a binary system is by the Flory-Huggins interaction parameter, where the thermodynamic miscibility of the two components is investigated to study their phase stability (6, 160). Another method to observe the compatibility of two components for amorphisation is by testing their likelihood for salt formation. The difference in acid dissociation constant in logarithmic form,  $\Delta pK_a$  suggests the likelihood of strong intermolecular bonds between VAL and NIC. Table 2.3 shows the pK<sub>a</sub> values of the two components as well as the  $\Delta pK_a$  values of their binary system. For a system to form a salt, the  $\Delta pK_a$  must be greater than zero and a value less than zero indicates the potential formation of a co-crystal or co-amorphous system (10). The  $\Delta pK_a$  values of the investigated VAL-NIC system are less than zero, hence the formation of hydrogen bonding is expected, and no salt formation is expected to occur within the systems, making them suitable for co-amorphisation.

### 2.5.3. Preparation Methods

Numerous lab scale methods are reported for the preparation of co-amorphous systems. These preparation methods can be classified into three different methods, namely, thermal preparation, solvent evaporation, and mechanical processing.

Lim *et al.* investigated the different processing techniques of ball milling, solvent casting, and quench cooling for preparation of co-amorphous systems of indomethacin and naproxen as the APIs with cimetidine as the co-former (37). It was concluded that the different preparation methods resulted in varying recrystallisation tendencies of CASs, thus indicating the influence of processing technique on the co-amorphous systems. Various properties of drug and excipients like thermal stability, melting point and crystallisation tendency influence the selection of preparation method. Table 2.4 presents

some examples of drug-drug CASs and drug-excipient CASs prepared by these techniques.

### 2.5.3.1. Solvent Evaporation

The solvent evaporation technique is used in the preparation of CASs. There are many ways to perform solvent evaporation, but the four most popular ways are freeze drying, spray drying, solvent casting and rotary evaporation as depicted in Figure 2.4.

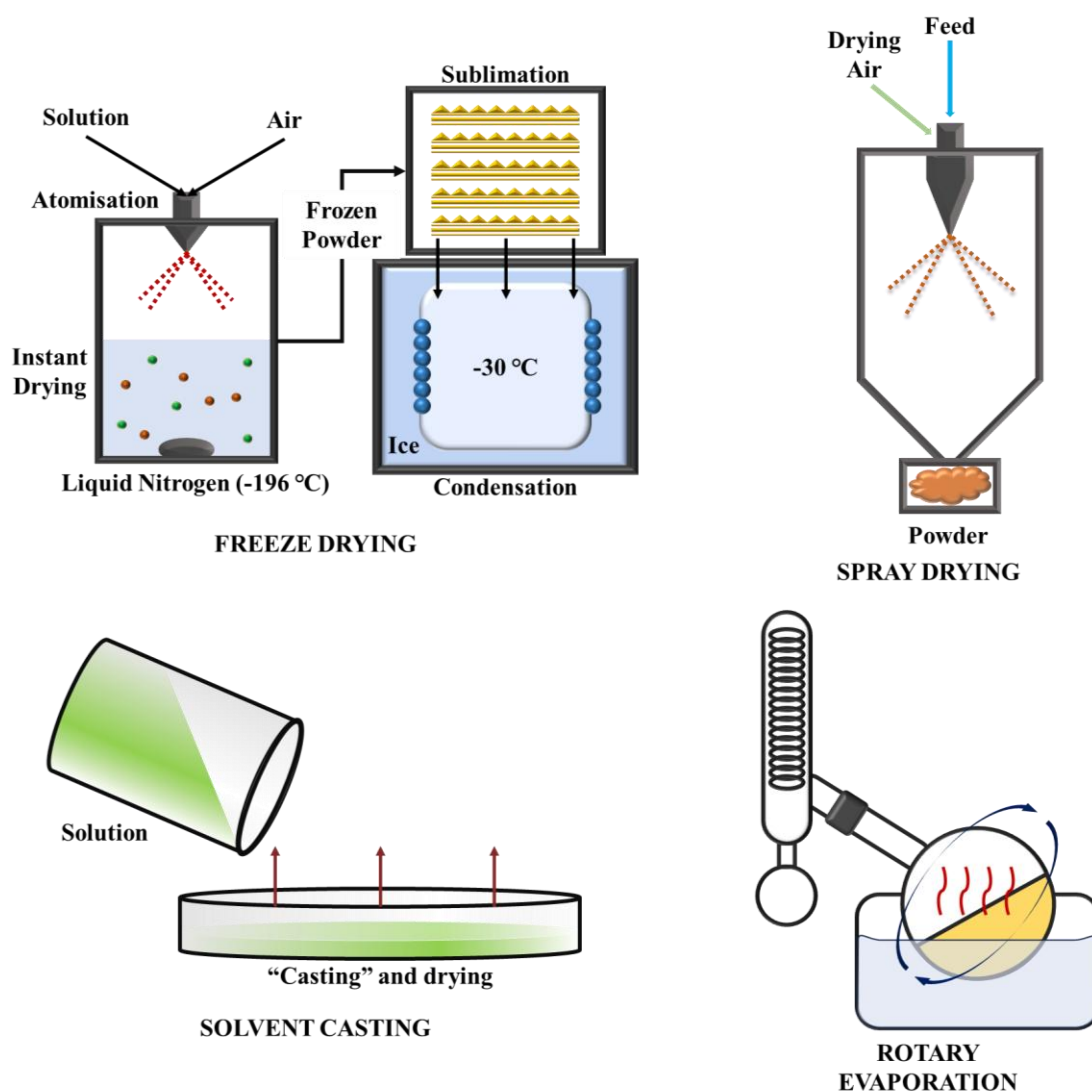


**Figure 2.4:** Different solvent evaporation processing techniques.

#### 2.5.3.1.1. Spray Drying

Spray drying is a widely used preparation technique to develop amorphous drug products. It is a single-step process of preparing fine, dry powders from solutions by

rapidly transforming them into fine droplets simultaneously while drying them with hot air, thus saving time.

This technique successfully prepares CASs with improved performances. Jensen *et al.* prepared equimolar CASs of indomethacin with lysine, arginine, and histidine from an acetone-water solution by spray drying, all of which maintained the amorphous state for 10 months when stored at 40 °C under dry conditions, with improved dissolution rates for all the systems (36). Dipyridamole and dipeptide aspartame was successfully transformed into a CAS from a methanol-water solution, from a series of co-formers, where the rest of co-formers failed to amorphise, as evidenced by crystalline peaks in their pXRD patterns. (27). However, it was very unstable, with the CAS recrystallising within a day of storage at 60% RH at 25 °C. This would suggest that the systems prepared by spray drying may be amorphous but may not be stable against recrystallisation when stored in a moisture-laden environment.

Moreover, the physical stability of the CASs prepared by this technique varies from one system to the other and are often dependent on the parameters of the spray dryer. Lens *et al.* produced a CAS of indomethacin with arginine by spray drying but the system undergoes phase separation, detected by DSC, which was caused by the evaporation rate of the solvent mixture of acetone and water at a temperature of 40 °C. This is a temperature below the boiling point of acetone (56 °C) and is used to prevent accidents (35). Beyer *et al.* also observed that variations in the parameters of the spray drying technique affect the physical stability of the CAS of naproxen and indomethacin, where systems obtained from highest flow rate but lowest inlet temperature recrystallised after 28 days followed by the system with the highest inlet temperature and lowest flow rate which was stable against recrystallisation (by pXRD and FT-IR) for longer than 28 days under dry conditions. This is thought to be because the lower inlet temperatures could potentially leave behind solvent residues that aid recrystallisation (161). However, the system prepared by intermediate flow rate at an intermediate inlet temperature was stable the longest, as it lacked the thermal stress higher temperatures posed on the system along with a low quantity of residual solvent in the spray dried system, thereby indicating that both temperature and flow rate affect the recrystallisation tendencies of the CASs (161).

Spray drying is an excellent method of preparing co-amorphous formulations but there is a need to investigate the long-term stability of these systems against recrystallisation to identify how these various drug systems behave under varying

environmental conditions. For this project, the parameters of the spray dryer were kept fixed for the preparation of the CASs to avoid batch to batch variation and the methodology has been discussed in [Chapter 3](#).

#### 2.5.3.1.2. Freeze Drying

Freeze drying or lyophilisation is low-temperature dehydration technique, where the components in a solution are atomised into liquid nitrogen causing them to freeze instantly, due to the glass forming ability of the components (162). The suspension is then subjected to sublimation at a very low temperature and pressure. This sublimation can take place within a few hours or several days, which is then followed by desorption where moisture is removed by increasing the temperature under high vacuum and the moisture travels to the condenser, thus leaving behind a dry product (163).

Alsalmi *et al.* successfully prepared CASs of indomethacin with tryptophan by using a *tert*-butyl alcohol (TBA)-water solvent mixture (164). The systems were prepared by varying mixtures of indomethacin with TBA and tryptophan with water, which was then subjected to freeze drying, thus forming three different kinds of systems, namely indomethacin-TBA, tryptophan-water and their mixtures of 30 to 90% w/w drug. The drug-excipient mixture was amorphous across the compositional space and enhanced drug release for systems with 40-60% drug content. However, the system with highest indomethacin content recrystallised by pXRD after 6 months at 5% RH at 25 °C, whilst systems with 50-80% of indomethacin maintained the amorphous state for over 9 months with mild crystallinity observed by pXRD that can be attributed to tryptophan, indicating that the drug remained amorphous. When the stability studies were conducted at 75% RH at 25 °C, the CASs became a sticky mass and recrystallised within a month by pXRD.

Wostry *et al.* prepared CASs of naproxen with arginine and lysine by freeze drying in combination with different surfactants, to enhance the drug's solubility (165). It was observed that the systems prepared in the absence of surfactants had poor stability against recrystallisation, which occurs even in dry conditions within 4 to 8 weeks. The incorporation of some surfactants extended the stability up to 18 weeks. Lysine as the co-former had more stability than arginine, as CASs of naproxen and arginine had higher crystallisation tendencies as the solid content increases. This study was unable to establish a connection between recrystallisation in the CAS with the presence of surfactants. Although freeze drying was able to produce CASs, the stability of the systems was poor regardless of the presence or absence of surfactants under dry conditions.

Although freeze drying is capable of preparing CASs, it takes multiple steps and is time consuming. Furthermore, recrystallisation occurs readily within the systems with or without high moisture content in its surroundings. This recrystallisation might be due to the presence of trapped moisture in the systems that was not completely eliminated during desorption. With multiple time-consuming steps for preparation combined with varying outcomes in the long-term stability of the CASs, freeze drying may not be best suited technique to prepare drug formulations swiftly for stability studies at the laboratory scale.

#### 2.5.3.1.3. Rotary Evaporation

One of the most commonly used lab scale techniques to prepare CASs. It is a technique to prepare co-amorphous systems where the solution containing the components are placed in a round bottom flask, rotated by a motor in a heated bath under reduced pressure to facilitate rapid evaporation, with a condenser and a separate flask to collect the evaporated solvent, and leaving fine dry powder in the rotating flask.

Madgulkar *et al.* prepared CASs of rilpivirine and nicotinamide at molar ratios of 2:1, 1:1, and 1:2 by rotary evaporation by evaporating methanol and quench cooling (166). It was observed that not only did the equimolar system prepared by rotary evaporation have higher solubility, higher drug release and higher dissolution compared to the system prepared by quench cooling but also the CASs prepared by rotary evaporation retained an amorphous state for 3 months when stored at 75% RH at a temperature of 40 °C. This enhanced performance and stability can be attributed to the miscibility between the components lowering the tendency to phase separate (166). Turek *et al.* successfully prepared equimolar CAS of valsartan and nicotinamide by rotary evaporation by evaporating ethanol and also by combining it with ball milling, both of which maintained an amorphous state for 9 and 11 months respectively, as evidenced by the lack of crystalline peak in the pXRD patterns, when stored at 25 °C and 0% relative humidity (10). Srinivas *et al.* prepared CASs of raloxifene HCl with naringin by rotary evaporation of an 80% v/v ethanol-water mixture in the ratios of 2:1, 1:1, and 1:2 (32). Across the compositional space, the solubility and dissolution for raloxifene HCl increased significantly for the CASs; the functionalities of naringin were not explored, i.e., only performance of the raloxifene HCl in terms of solubility and dissolution was reported. However, the stability studies were restricted to dry conditions. The systems retained an amorphous state for 2 years when stored at 0% RH at 25 °C.

The outcome for performance and stability studies of systems prepared by rotary evaporation often varies across systems. Wang *et al.* attempted to prepare co-amorphous systems of valsartan and 2-aminopyridine at molar ratios of 2:1, 1:1 and 1:2 by rotary evaporation (34). CASs with the molar ratios 1:1 and 1:2 were amorphous but the 2:1 system demonstrated some crystallinity by pXRD. However, the 1:2 system had exceedingly high solubility compared to the equimolar system, a 15.6-fold increase and 3.5-fold increase respectively, in comparison to pure VAL and the dissolution rate was higher in the CAS with higher amount of excipient, although all the CASs had improved dissolution profiles compared to the drug itself. The stability studies conducted at room temperature under 0% RH demonstrated that the system with the higher amount of excipient retained an amorphous state for 8 months, but the equimolar system recrystallised, with pXRD peaks appearing from both the components during that same time period. It was assumed that the equimolar system would be most stable against humidity due to the uniform distribution of components that would enable strong bonding interactions between the component without any steric hinderances, possibly due to multiple bonding sites. This might make them stable against phase separation and ultimately recrystallisation. The outcome from the study by Wang *et al.* however suggests that equimolar systems may not always be the best formulation in terms of performance and stability.

It is obvious that the investigation on the stability and performance of CASs when stored at high humidity has not been well explored, even for systems prepared by rotary evaporation. This presents the opportunity to investigate the influence of high moisture content in the environment in which CASs are stored.

#### 2.5.3.1.4. Solvent Casting

Solvent casting is another solvent evaporation method where a drug and a secondary component are dissolved in a solvent to ensure mixing, followed by ‘casting’ the solution over a flat surface, and the solvent gets evaporated, either under heat, pressure or even at ambient conditions. This creates an amorphous film (which can be broken down by a mortar and a pestle), with enhanced solubility and dissolution for poorly soluble drugs. This technique is ideal for early-stage screening requiring minimal amount of starting materials, making way for quick assessment of stability and performance of the drug delivery system prior to large scale preparation methods.

Usually, this method is applied to drug delivery systems with polymers, but CASs have been prepared by this method as well. Gao *et al.* has employed solvent casting to prepare CASs of repaglinide and saccharine at multiple molar ratios (3:1, 2:1, 1:1, 1:2 and 1:3) (42). The CASs were subjected to vacuum drying to remove residual solvents and after 2 days of evaporation at ambient conditions, where only CASs of molar ratios 3:1, 2:1 and 1:1 were completely dry while the remaining two CASs remained semi-solid masses even after more than 12 hours of subsequent vacuum drying. Only the equimolar system was a homogeneous, single-phase system as evidenced by a singular endothermic event of a glass transition by DSC thermograms and having an enhanced dissolution profile with respect to the API. A major disadvantage of the solvent casting method is that it is time consuming. Furthermore, there is an added step of removing residual solvent, making this preparation technique more tedious than rotary evaporation. CASs of valsartan and vanillin in the ratios 2:1, 1:1 and 1:2 were prepared by Ali *et al.* by a modified form of solvent casting, where the systems were subjected to ambient drying conditions and further stored in a vacuum desiccator for final solvent removal (38). Only the equimolar system was able to produce an amorphous powder, but the two other systems formed a sticky mass, hence were rejected from further analysis. The equimolar system did show enhanced solubility and an enhanced rate of dissolution compared to the drug itself but a long-term study of the CAS under high humidity was not explored. In this method of preparing CASs, the influence of varying molar ratios on performance cannot be explored as an excess of the components yields semi-solid masses instead of powders.

Although it is good as a screening process to prepare CASs, it is not the best method to prepare CASs on a large scale. Additionally, the presence of solvent residues may create stability problems with regards to recrystallisation or solvate formation during storage. Solvent removal rate and temperature have a significant effect on stability, dissolution and particle size of the CAS and hence need careful evaluation. In addition, low yield, and high cost of recovery of solvent poses economic limitations on this method, making it an unsustainable method of preparation for commercial pharmaceutical formulations.

### 2.5.3.2. Mechanical Processes

Mechanical processing is a solvent-free or low-solvent process that can be used to prepare co-amorphous systems and there are of two types, namely milling (ball milling

and cryo-milling) and manual grinding (solvent-assisted grinding) as demonstrated in Figure 2.5.

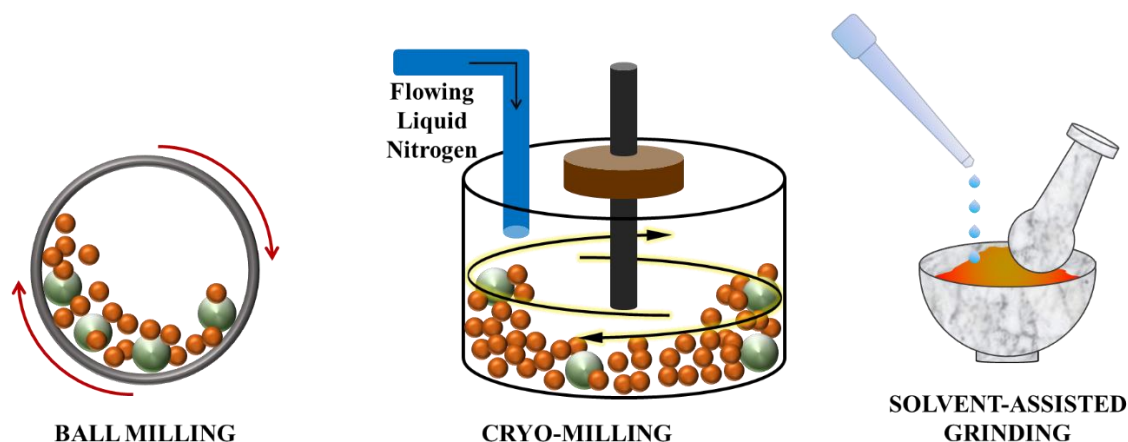


**Figure 2.5:** Different mechanical processing techniques.

#### 2.5.3.2.1. Ball milling and Cryo-milling

Although milling can prepare CASs with enhanced solubility and dissolution, the stability and performance of binary systems in different storage conditions can be independent of the mode of preparation. It may be that irrespective of the milling process, the products do not fully mix and are subjected to phase separation and eventually recrystallisation over time.

Ball milling is a mechanochemical, grinding technique that utilises milling media such as steel or ceramic balls within a grinding vessel, where the vessel is shaken in varying motions, depending on the type of equipment, to reduce the size of the starting materials into finer particles. The mechanical process of grinding mixes the components at a molecular scale and initiates chemical reaction between the components. The use of low solvent or the lack of it assists in reducing the additional energy of removing solvents, making it a greener approach for preparing amorphous powder (167). During the formation of co-amorphous formulations, these high energy collisions within the ball mill disrupts the bonding interaction within a crystal lattice of individual starting components and promotes mixing, making them susceptible to new intermolecular interaction between the components, thus potentially forming a binary system. This binary system can possess a disordered state with enhanced solubility as the crystal lattice has been disrupted due to the mechanical impacts.

Cryo-milling follows the same principle as ball milling. In cryo-milling, the components are cooled by equipping with liquid nitrogen at  $-196\text{ }^{\circ}\text{C}$  (168). The cooled

materials are then subjected to spinning that results in high energy impacts from the balls in the mill, which shatters down the material into fine, homogeneous powder. Cooling at a temperature below the component's  $T_g$  makes it brittle due to its glass forming ability, thereby reducing molecular mobility, and inhibiting crystallisation.

Wairkar and Gaud prepared CASs of nateglinide and metformin HCl by ball milling in the ratios of 1:1, 1:3, 1:5 and 6:25 (dose ratio) (43). Solubility of the systems increased with increasing co-former content, with the solubility increasing by 7 times in the lowest dose ratio. Furthermore, the shifts in the peak position in the FT-IR spectra of the functional groups of the individual components of the CASs indicate the presence of hydrogen bonding interactions, which stabilise the binary system. However, during storage in 75% RH at 40 °C, nateglinide maintained its amorphous state but the co-former recrystallised, indicating that the stability of the CASs against recrystallisation relies on the crystallisation tendency of the co-former metformin HCl in the CAS. Löbmann *et al.* successfully prepared multiple equimolar CASs with indomethacin and carbamazepine with different amino acids by ball milling, with enhanced dissolution as evidenced by HPLC data and strong intermolecular interactions as evidenced by DSC data (28).

The bonding interaction is often not detected in CASs prepared by milling. Löbmann *et al.* prepared single phase homogeneous CASs of simvastatin and glipizide at molar ratios of 2:1, 1:1, and 1:2 by cryo-milling and 2:1 and 1:1 by ball milling (6). However, the presence of melting endotherms in the DSC data indicates the presence of low levels of crystallinity in the CASs prepared by both the methods. The presence of crystallinity would suggest that the system lacks homogeneous bonding interaction between the system, which is supported by the FT-IR data where no hydrogen bonding interaction could be detected. Although all the CASs demonstrated improved solubility and dissolution, their stability against recrystallisation varied with the modes of preparation. Recrystallisation occurred more readily within a few days (8 to 16 days) in ball milled systems when stored at 25 °C/60% RH, as evidenced, as peaks shift in FT-IR spectra of some functional groups of the CASs to that of the starting components in their crystalline forms along with the reappearance of the crystalline peaks in the pXRD patterns, but maintained their amorphous state for over four months when stored at 4 °C/0% RH. For cryo-milled CASs stored at 25 °C/60% RH, they have higher stability against crystallisation tendencies, maintaining their amorphous states for 46, 74 and 74 days (2:1, 1:1 and 1:2 systems by pXRD). However, when those stored at 4 °C/0% RH retained their amorphous states for approximately 3 months when, compared to the BM

systems of the same composition. Systems stored at 25 °C/0% RH showed similar levels of stability against recrystallisation, across the compositional space, irrespective of their preparation techniques. The higher stability of cryo-milled CASs in higher humidity is possibly due to the better starting material prior to the ageing studies, where the systems were prepared in an dry, inert environment due to the displacement of oxygen by the liquid nitrogen as well as creating a dry cold environment that reduces moisture adsorption in the systems, along with very little thermal degradation due to the low processing temperature (169). The lack of bonding interaction was observed again when Renuka *et al.* attempted to prepare CASs of two BCS class II drugs, glipizide and atorvastatin using cryo-milling in varying w/w ratios (1:1, 1:2, and 2:1) for the treatment of hyperlipidaemia and hyperglycaemia (41). Although the CASs with the ratios 1:1 and 2:1 were X-ray amorphous and a single glass transition event was present, it was not entirely amorphous because of the additional presence of melting peak endotherms for both the components. There was a significant improvement in dissolution profile but no interactions between the components were observed by Raman spectroscopy. Although the equimolar system was stable for over 6 months when stored at 75% RH at 40 °C, the 2:1 CAS had recrystallised within 3 months.

Zou *et al.* observed that equimolar CASs between different BCS class II drugs with different excipients prepared by ball milling are essentially amorphous by pXRD for almost 3 months in all systems, with some exceptions (39). However, the dissolution rate can increase or decrease over time, despite a lack of moisture in the storage environment, potentially due to amorphous-amorphous phase separation as evidenced by multiple  $T_g$ s after storage. This indicates that the CASs undergo temporal phase separation which may affect their performance in the long run.

Although milling can prepare CASs with enhanced solubility and dissolution, the stability and performance of binary systems can be independent of the mode of preparation with respect to their storage conditions. It may be that they are subjected to phase separation and eventually recrystallisation over time. To explore the long-term effects of ball milling, this processing method has been included in this project to prepare co-amorphous systems of valsartan and nicotinamide, and it is discussed in [Chapter 3](#).

#### **2.5.3.2.2. Solvent-Assisted Grinding (manual grinding)**

Solvent-assisted grinding is a manual, mechanochemical process where different components are subjected to mechanical impacts such as grinding with a small quantity

of solvent to initiate chemical reaction between the components. This method combines solvent-based and mechanically-induced reaction to prepare drug products (170).

Although this method is simple, it is time consuming and does not produce products with the desired outcome. Turek *et al.* employed this method to prepare CASs of VAL and NIC by grinding them together with 6 drops of ethanol for 30 minutes and 60 minutes, but the resulting systems were not amorphous as evidenced by the crystalline peaks in the pXRD patterns (10). Skienh *et al.* attempted to prepare CASs between curcumin and folic acid dihydrate by this method but were only able to produce a semi-amorphous system after 90 minutes of grinding with 5 drops of methanol, as evidenced by the presence of a crystalline peak in their pXRD pattern (171).

Both of these examples indicate that this method is not a suitable technique for co-amorphisation.

### 2.5.3.3. Thermal Preparation

Thermal preparation of co-amorphous formulations involves heating a drug and co-former together, so that they melt together followed by rapid cooling to ensure that the molecules are frozen in a disordered state to form a single-phase, amorphous system. The thermal method of preparation is of two types, namely quench cooling and hot melt extrusion, as depicted in Figure 2.6.

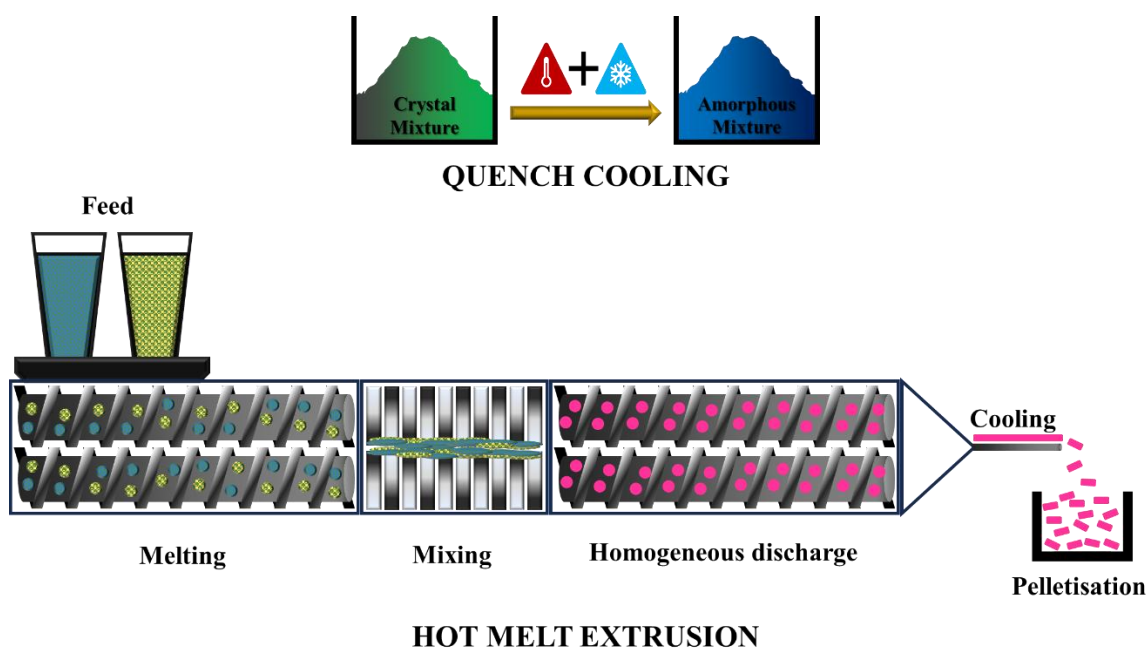


Figure 2.6: Thermal preparation techniques.

#### 2.5.3.3.1. Hot melt extrusion

Hot melt extrusion is a thermal, solvent-free, continuous processing technique which can be scaled up. In a single continuous process, the materials are melted at high temperatures and mixed in a screw extruder at a molecular level thus producing a homogeneous molten mass. It is then extruded out through a die and cooled in the downstream processing and divided into smaller shapes and sizes as desired, such as pellets. Hot-melt extrusion has proven highly efficient in producing an amorphous blend of active ingredients such as curcumin and piperine with Soluplus®, evidenced by a lack of melting endotherms in the DSC data, enhancing the bioavailability and biological efficacy (172).

Lenz *et al.* successfully implemented hot melt extrusion to prepare CASs of indomethacin and arginine, with and without polymers (35). In this study, spray drying was also employed to prepare CASs. Although there were no significant differences in their performances, the extruded product underwent phase separation, which was detected by DSC. The binary extruded products were amorphous for 6 months and the binary spray dried products maintained amorphisity by pXRD for 18 months under dry conditions at 23 °C and 40 °C. Although spray drying produced a more shelf-stable system, this study did establish that it is possible to prepare CASs by hot melt extrusion at a small scale, as this technique has always been utilised for preparing pharmaceutical products with polymers. This technique was utilised by Arnfast *et al.* to successfully prepare equimolar CAS of indomethacin and cimetidine, which underwent amorphous-amorphous phase separation after 4 weeks of storage at low relative humidity of 11% at 25 °C, evidenced by the appearance of two glass transition events by DSC (101).

This method of preparation has not been the most successful for the development of stable co-amorphous formulations; another thermal technique, namely quench cooling can be explored to prepare these binary formulations.

#### 2.5.3.3.2. Quench cooling

Quench cooling is a popular solvent-free technique to prepare small batches of amorphous formulations of pharmaceutical products. Quench cooling works by combining an API with suitable co-formers and heating them beyond their melting point to form a homogeneous melted mixture. This melted mass is then subjected to rapid cooling by either using liquid nitrogen (-196 °C) or bringing them to a lower temperature (0 °C to 30 °C). This rapid cooling prevents recrystallisation, maintains the glassy state and thus forming a co-amorphous product (170).

Löbmann *et al.* prepared CASs of indomethacin and naproxen in the molar ratios of 2:1, 1:1 and 1:2 with enhanced dissolution and chemical release by quench cooling (33). The equimolar system retained an amorphous state by pXRD at 4 °C and 25 °C under dry conditions for 21 days whereas systems with an excess of either of the components recrystallised readily at 25 °C. Although hydrogen bonding interactions stabilised the binary system, as evidenced by shift in peaks corresponding to the functional groups involved in hydrogen bonding in the FT-IR spectra, the systems failed to maintain an amorphous state in the absence of moisture, signifying temporal phase separation in systems with excess of the component that is potentially unbonded in the system. Madgulkar *et al.* utilised quench cooling to prepare multiple CASs of rilpivirine and nicotinamide but stability against recrystallisation was not explored under dry nor moist conditions (166).

Kilpeläinen *et al.* implemented a modified form of quench cooling (systems were cooled at room temperature) to prepare equimolar CASs of multiple BCS Class II drugs with different excipients, by melting them and cooling them rapidly at room temperature (173). Although the systems were not entirely amorphous before phase separation studies were conducted, as evidenced by the appearance of a melting event in the DSC data, FT-IR was able to identify phase separation in the CASs due to the changes in peak positions and intensities, which increased with time, and the evolution of phase separation varied from system to system. This study highlights that this mode of preparation may influence the stability of different systems differently. Furthermore, the low effort and low cost of this mode of preparation makes it a rapid screening technique when exploring multiple systems.

Although this method of preparing co-amorphous systems can only produce small batches whilst using liquid nitrogen to inhibit the crystallisation tendencies, it is successful in preparing CASs with excellent performance. Due to the ease and swiftness of this mode of preparation, this technique can easily act as a rapid screening method to test the viability of co-amorphisation of different components. Although the long-term stability studies of CAS made by this mode of preparation are not well explored. In this project, a modified form of quench cooling has been implemented to prepare co-amorphous formulations of valsartan and nicotinamide to not only test their long-term stability and performance but also to identify the most apposite molar ratio to prepare CASs of these two components and this is explored in Chapters 3 to 6.

**Table 2.4:** Examples of binary co-amorphous drug delivery systems of BCS Class II drugs.

| <u>Co-amorphous Formulations</u> | <u>Systems</u>                             | <u>Mode of Preparation</u>                    | <u>Ratios</u>                | <u>Characterisation Techniques</u> | <u>Observation</u>                                                                                                                                                                                                                                                                                                        |
|----------------------------------|--------------------------------------------|-----------------------------------------------|------------------------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Drug-Drug CAS</b>             | Nateglinide (NT) – Metformin HCl (MF) (43) | Ball Milling                                  | 1:1,<br>1:3,<br>1:5,<br>6:25 | pXRD, DSC, FT-IR, HPLC, SEM        | <ul style="list-style-type: none"> <li>• NT demonstrated enhanced solubility and dissolution in the dose ratio; no significant changes were not demonstrated for MF</li> <li>• FT-IR confirms the formation of bonding interaction</li> <li>• MT recrystallisation was observed for 45 days at 40 °C/75% RH</li> </ul>    |
|                                  | Simvastatin – Glipizide (6)                | Ball Milling (BM),<br>Cryomilling (CM)        | 2:1,<br>1:1,<br>1:2          | pXRD, DSC, TGA, FT-IR, HPLC,       | <ul style="list-style-type: none"> <li>• Single phase system in all ratios, with improved solubility, dissolution</li> <li>• Recrystallisation occurred more readily in CM CAS than BM CAS at 4 °C/0% RH; CASs prepared by CM were more stable at 25 °C/60% RH</li> </ul>                                                 |
|                                  | Glipizide – Atorvastatin (41)              | Cryomilling                                   | 2:1,<br>1:1,<br>1:2          | pXRD, DSC, Raman, SEM, HPLC        | <ul style="list-style-type: none"> <li>• Complete amorphisation not observed due to the presence of T<sub>m</sub> peaks</li> <li>• At 40 °C/75% RH, 1:1 stayed amorphous for more than 6 months but 2:1 recrystallised in 3 months</li> </ul>                                                                             |
|                                  | Naproxen – Indomethacin (33)               | Quench Cooling                                | 2:1,<br>1:1,<br>1:2          | pXRD, DSC, FT-IR, HPLC             | <ul style="list-style-type: none"> <li>• 1:1 system staid amorphous at 4 °C and 25 °C under <i>dry conditions</i> for 21 days; ratio with excess of the APIs demonstrated recrystallisation</li> <li>• H-bonding interaction identified with FTIR</li> <li>• Improved dissolution and chemical release in CASs</li> </ul> |
|                                  | Raloxifene HCl – Naringin (32)             | Rotary Evaporation (ethanol–H <sub>2</sub> O) | 2:1,<br>1:1,<br>1:2          | pXRD, DSC, FT-IR, NTA, DVS,        | <ul style="list-style-type: none"> <li>• Moisture sorption was 3 times higher for CASs than the API raloxifene</li> <li>• Solubility and dissolution increased 3.5 times and 10 times in CASs</li> <li>• All CASs remained amorphous for 2 years when stored at 25 °C/0% RH</li> </ul>                                    |

|                           |                                                                                                                                                          |                                                |     |                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|-----|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                           | Cimetidine – Captopril/ Nizatidine/<br>Perphenazine<br><br>Clotrimazole – Piroxicam<br><br>Indomethacin –<br>Clotrimazole/Paracetamol/Piroxicam<br>(173) | Melt<br>Amorphisation                          | 1:1 | DSC, FT-IR                                  | <ul style="list-style-type: none"> <li>Amorphous-Amorphous Phase Separation was detected by high resolution imaging FT-IR in every CAS, which <i>increased with time</i></li> <li>Spectral data was analysed by Principal Component Analysis</li> <li>Some samples thickened during storage, which increased absorption and led to the saturation of their IR spectra and thus eliminated</li> </ul>                                                                                                          |
|                           | Indomethacin – Cimetidine (101)                                                                                                                          | Hot Melt<br>Extrusion                          | 1:1 | tmDSC, DVS                                  | <ul style="list-style-type: none"> <li>Temporal phase separation was observed in the CAS after 4 weeks when stores at 25°C/11% RH</li> </ul>                                                                                                                                                                                                                                                                                                                                                                  |
| Drug-<br>Excipient<br>CAS | Carbamazepine – Tryptophan<br><br>Indomethacin –<br>Arginine/Phenylalanine/Tryptophan<br>(28)                                                            | Ball Milling                                   | 1:1 | pXRD, tmDSC,<br>HPLC, UV-VIS                | <ul style="list-style-type: none"> <li>Carbamazepine only produced a CAS with tryptophan, whereas indomethacin was successfully amorphised with the three amino acids</li> <li>Tyrosine failed to produce CAS for both APIs</li> <li>APIs were chemically stable after the milling process</li> <li>CASs were amorphous for over 6 months at 40 °C under <i>dry</i> conditions</li> <li>Improved dissolution rate for all CASs with respect to the API both in its amorphous and crystalline form.</li> </ul> |
|                           | Indomethacin –<br>Histidine/Arginine/Lysine<br>(36)                                                                                                      | Spray Drying<br>(acetone- H <sub>2</sub> O)    | 1:1 | pXRD, tmDSC,<br>FT-IR, HPLC,<br>UV-VIS, SEM | <ul style="list-style-type: none"> <li>Increased T<sub>g</sub> for all CASs</li> <li>No recrystallisation for over 10 months at room temperature and 40°C at <i>low humidity</i></li> <li>Improved dissolution under gastrointestinal conditions (pH 7.2, 37 °C)</li> </ul>                                                                                                                                                                                                                                   |
|                           | Naproxen – Arginine/Lysine (165)                                                                                                                         | Freeze Drying<br>(Surfactant<br>based solvent) | 1:1 | pXRD, tmDSC,<br>TGA                         | <ul style="list-style-type: none"> <li>Amorphisity depended on surfactants</li> <li>Without surfactants, CASs recrystallised within 4 to weeks at room temperature under <i>dry conditions</i>.</li> </ul>                                                                                                                                                                                                                                                                                                    |

|  |                                  |                                                                                          |                     |                                             |                                                                                                                                                                                                                                                                                                                                                          |
|--|----------------------------------|------------------------------------------------------------------------------------------|---------------------|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Valsartan – Nicotinamide (10)    | Ball Milling,<br>Rotary<br>Evaporation<br>(ethanol),<br>Solvent-<br>Assisted<br>Grinding | 1:1                 | pXRD, DSC, FT-<br>IR, solid-NMR,<br>SEM     | <ul style="list-style-type: none"> <li>• Single phase system ball milled and rotary evaporated CAS</li> <li>• 3.5 times increase in aqueous solubility</li> <li>• Identification of modes of hydrogen binding</li> <li>• No recrystallisation for at least 9 (rotary evaporation) and 11 (ball milling) months at 25 °C at 0% RH</li> </ul>              |
|  | Valsartan – Vanillin (38)        | Solvent Casting<br>(ethanol)                                                             | 2:1,<br>1:1,<br>1:2 | pXRD, DSC, FT-<br>IR, HPLC, SEM             | <ul style="list-style-type: none"> <li>• Only 1:1 produced amorphous powder; rest formed a sticky mass</li> <li>• Improved solubility, dissolution, and drug loading efficiency</li> <li>• Stability studies under high humidity was not explored.</li> </ul>                                                                                            |
|  | Rilpivirine – Nicotinamide (166) | Rotary<br>Evaporation<br>(RE)<br>(methanol),<br>Quench Cooling<br>(QC)                   | 2:1,<br>1:1,<br>1:2 | pXRD, DSC, FT-<br>IR, HPLC, UV-<br>Vis, SEM | <ul style="list-style-type: none"> <li>• Increased solubility, dissolution, permeation, and oral bioavailability</li> <li>• Solubility increased with the increase in NIC concentration</li> <li>• RE CASs show better solubility and dissolution compared to QC CAS</li> <li>• RE CASs remained amorphous after 3 months in 40±2 °C/75±5% RH</li> </ul> |
|  | Minoxidil – citric acid (26)     | Solvent Casting<br>(methanol)                                                            | 1:1,<br>1:2,<br>1:3 | pXRD, DSC, FT-<br>IR, HPLC, UV-<br>Vis, SEM | <ul style="list-style-type: none"> <li>• CAS formation across all ratios with higher solubility and dissolution</li> <li>• Stability studies under high humidity were not explored.</li> </ul>                                                                                                                                                           |
|  | Dipyridamole – Aspartame (27)    | Spray Drying<br>(methanol–<br>H <sub>2</sub> O)                                          | 1:1                 | pXRD, tmDSC,<br>FT-IR, TGA, HS,<br>DVS      | <ul style="list-style-type: none"> <li>• CAS recrystallised in one day when 25 degrees C/60 % RH conditions.</li> </ul>                                                                                                                                                                                                                                  |

|  |                                                                                                                                   |                                                               |               |                                    |                                                                                                                                                                                                                                                                                                                                                                                |
|--|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|---------------|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Valsartan – 2-Aminopyridine (34)                                                                                                  | Rotary Evaporation (methanol)                                 | 2:1, 1:1, 1:2 | pXRD, DSC, FT-IR, Raman, HPLC      | <ul style="list-style-type: none"> <li>Only 1:1 and 1:2 CASs were amorphous with the latter having enhanced solubility and higher <math>T_g</math> value</li> <li>1:1 CASs recrystallised after 8 months after storage in 0% RH condition under room temperature; 1:2 retained its amorphous state</li> </ul>                                                                  |
|  | Valsartan – Biotin/Arginine/Tryptophan<br>Tadalafil – Biotin/Arginine/Tryptophan<br>Mebendazole – Biotin/Arginine/Tryptophan (39) | Ball Milling                                                  | 1:1           | pXRD, TGA, tmDSC, FT-IR, HPLC      | <ul style="list-style-type: none"> <li>All CASs except VAL-Biotin maintained their amorphous form when stored at dry conditions for 80 days (biotin recrystallises)</li> <li>The drug-co-former molecular interactions showed no relationship with the IDR changes (increase and decrease) of co-amorphous systems during storage, possibly due to phase separation</li> </ul> |
|  | Indomethacin – Arginine (35)                                                                                                      | Hot Melt Extrusion (HME), Spray Drying (SD)                   | -             | pXRD, tmDSC, FT-IR, HPLC, DVS      | <ul style="list-style-type: none"> <li>HME technique was used to prepare CAS for the first time (successfully)</li> <li>Phase separation occurred during HME</li> <li>Dissolution rate of the CASs was similar for HME and SD</li> <li>HME CAS and SD CAS were stable for 6 and 18 months at 23°C and 40°C under <i>dry conditions</i>.</li> </ul>                             |
|  | Indomethacin – Tryptophan (164)                                                                                                   | Freeze Drying ( <i>tert</i> -butyl alcohol– H <sub>2</sub> O) | -             | pXRD, DSC, TGA, UV-Vis, FT-IR, NMR | <ul style="list-style-type: none"> <li>When stored at 25±2 °C/75±5% RH, CASs recrystallise within a month</li> <li>CASs with 50-80% w/w stayed homogeneous for over 9 months at 25±2 °C/5±5% RH</li> </ul>                                                                                                                                                                     |
|  | Repaglinide – Saccharine (42)                                                                                                     | Solvent Casting (methanol)                                    | 1:1           | pXRD, DSC, FT-IR, HPLC             | <ul style="list-style-type: none"> <li>Solubility enhancement by 20 times and enhanced dissolution</li> <li>No recrystallisation when stored at 40 °C/75% RH for over 3 months</li> </ul>                                                                                                                                                                                      |

## 2.6. Challenges in the formulation of co-amorphous systems

Co-amorphous systems enhance the performance of poorly soluble APIs and their bioavailability, but maintaining their physical stability against phase transformation is a challenge.

### 2.6.1. Phase separation and recrystallisation

It has already been established that APIs formulated in CASs have improved solubility and dissolution rates compared to the lone API, whether it is its crystalline or amorphous (40). The long term stability of the systems have often not been fully investigated, or if they were, these systems were stored under little to no humidity at 0 °C to room temperature, where they maintain an amorphous state for long periods of time, as evidenced by a lack of crystalline peaks in pXRD patterns (10, 26, 28, 32-39, 161). Some accelerated ageing studies have been conducted on co-amorphous systems where the systems are stored at relatively moderate to high humidity (60 to 75% RH) at temperatures of 0 °C, room (20 to 25 °C) and 40 °C (6, 27, 41-43). The recrystallisation pathways have not been explored extensively in these works beyond identification by pXRD data analysis.

However, for recrystallisation to occur, the co-amorphous systems would have to transform a single homogeneous phase to heterogeneous phases, by overcoming the secondary bonding interactions that are holding the single phase together. Kilpeläinen *et al.* was able to detect phase separation in 7 CASs of indomethacin and piroxicam, captopril and cimetidine, cimetidine and nizatidine, cimetidine and perphenazine, clotrimazole and indomethacin, clotrimazole and piroxicam, and indomethacin and paracetamol. FT-IR spectra of amorphous forms of clotrimazole and nizatidine (individually) were used to identify and measure relative changes in peak intensities and baselines on ageing where nizatidine recrystallised after 1 week of storage at a temperature range of 52 to 75 °C and clotrimazole showed very small changes in intensities with an increase in baseline over the same time (173). Amorphous-amorphous phase separation (AAPS) of the aged CASs was detected by principal component analysis of the spectral data of the CASs using the pure systems as a reference. AAPS took place differently, at different timepoints for all drug-drug CASs, indicating that every binary mixture can undergo AAPS. As such, AAPS can occur in dry conditions or in a moisture-laden environment, where multiple amorphous forms can exist, leading to a system where an API and co-former are existing freely in the system, thus disrupting the homogeneity

of the system (97, 174). Zou *et al.* investigated AAPS by investigating the  $T_g$ s of the CASs with tmDSC and studying the impact of AAPS on the performance of the CASs of three different APIs with three different excipients (Table 2.4) (39). Phase separated CASs of valsartan and tadalafil demonstrated a lowering of dissolution rate over time (at 0, 40 and 80 days) at low humidity for all the excipients but the phase separated CASs of mebendazole with excipients arginine and tryptophan showed a small increase in the dissolution rate, indicating that phase separation does not affect the performance of all drug systems the same way.

Due to the metastable nature of amorphous materials, it is evident that the mode of preparation of co-amorphous systems can influence these systems' characteristics and performance (175-177). The starting components are exposed to different experimental parameters such as temperature, stress, solvents of varying pH for the preparation of the final material, and all of these affect the miscibility of the starting components and by extension, the homogeneity of the final product, which ultimately affects the performance of the system in terms of stability, solubility, and dissolution (178, 179). Hence, it is important to explore the impact of different processing techniques on a model co-amorphous system to investigate its resistance against phase separation and recrystallisation.

Co-amorphous formulations destabilise due to disruption of secondary bond interactions, namely hydrogen bonding interactions or  $\pi$ - $\pi$  interactions, that stabilises these binary systems. The presence of moisture in the surroundings disrupts the intermolecular hydrogen bonds between the two components due to the interaction of water molecules with the hydrophilic functional groups of either the API or the co-former. Water molecules can also disrupt the  $\pi$ - $\pi$  interaction between the aromatic groups of the two components. A water molecule is small enough (1.4 Å in length, e.g. as reported in the Mercury software) to migrate between the aromatic rings stacked together in the  $\pi$ - $\pi$  bonding interaction, where the distance between the rings are approximately 3.3 to 3.8 Å, this will disrupt and can potentially break the stacked aromatic ring association, and further aid in the destabilisation of the CAS (146, 180, 181). The disruption of these secondary bonding interactions between the two components can lead to phase separation and ultimately recrystallisation, thus the presence of moisture in the surroundings can destabilise CASs more readily than a lack thereof (182). It is important to note during the development of co-amorphous systems, stability studies are necessary to be conducted at different relative humidity to investigate the long-term stability of these formulations

against AAPS, both moisture-induced or temporally occurring, and ultimately against recrystallisation, which begins with the formation of a stable crystal nucleus, brought about by nucleation.

### 2.6.2. Amorphous Hydrates

An amorphous hydrate can be defined as a water-saturated amorphous phase. In such a system water molecules can exist within the amorphous mixtures by bonding with components without forming any crystalline material. Due to the presence of two hydrogen donor atoms and two lone pairs of electrons, i.e., hydrogen bond acceptors, each water molecule can contribute towards forming a flexible tetrahedral network within an amorphous formulation, especially in co-amorphous formulations, as these systems are stabilised by hydrogen bonding interactions between functional groups (10, 28, 43, 164, 183).

For example, nicotinamide hydration within CASs (of varying ratios) of nicotinamide and prilocaine prepared by quench cooling has been shown to have a plasticising effect, increasing molecular mobility at room temperature that lowers  $T_g$  with the effect being most significant at the NIC-rich end of the CAS compositions (149). In addition, the possibility of co-amorphous formulations becoming hydrated mostly occurs when they are stored at high humidities, where there are enough water molecules to be adsorbed by the amorphous materials to form non-stoichiometric amorphous hydrates. This potentially causes degradation of chemical and physical stability (47). It is important to investigate how much the adsorption of moisture destabilises the metastability of the co-amorphous form.

## 2.7. Summary

The literature review presented here demonstrates the production of co-amorphous drug formulations with suitable co-formers and provides reasons why this might be beneficial. CAS production disrupts the regular arrangement of molecules in crystalline APIs and co-formers, and this can enhance the solubility and bioavailability of poorly soluble APIs. Although multiple CASs have been prepared by different methods, the influence of the processing routes on the long-term stability of these systems has not been widely explored. Producing pharmaceutical products with improved solubility, bioavailability and dissolution is not enough to transition this laboratory scale product development to large-scale manufacturing. Prior to scaling up, it is necessary to look into the long-term stability of these systems under different humidities, to understand

how the systems undergo phase transformation and ultimately recrystallisation, and how these transitions affect their performance.

## 2.8. Project Aim

The aim of this project is to prepare co-amorphous formulations of valsartan and nicotinamide by different processing routes and test the performance and long-term stability against recrystallisation under high humidity as well as testing the stability under dry conditions during that time.

## 2.9. Objectives

The specific objectives for this research with a link to each results chapter in which they are addressed are presented below:

- To use laboratory melt amorphisation technique for the preparation of VAL-NIC CASs to identify the most appropriate composition in terms of the least retained crystallinity, closest proximity to the theoretical glass transition temperature, aqueous solubility, and the longest stability against recrystallisation in storage at high humidity (results presented in [Chapter 4](#)).
- Upon the identification of the optimal VAL-NIC ratio, investigate the influence of different processing techniques on the CASs in terms of amount and type of amorphous versus crystalline phase content along with measurement of the solubility and dissolution rate (results presented in [Chapter 5](#)).
- Investigate the long-term stability against recrystallisation and phase separation of the optimal VAL-NIC CASs prepared by the different processing techniques by storing them under a range of humidity conditions (results presented in [Chapter 6](#)).

## Chapter 3. Materials and Methods

### 3.1. Chapter Overview

This chapter provides an overview of the materials and methodology that has been implemented for the preparation of the co-amorphous drug delivery systems and their characterisation along with a background on crystallisation and amorphisation. An outline of the techniques used will be provided along with a detailed methodology for data acquisition and interpretation of the data used in this thesis. A brief recap of the preparation methodology and the characterisation techniques will be included in the relevant experimental chapters to provide an easy overview to the reader. All the diagrams presented in this chapter have been prepared in Microsoft PowerPoint based on the images of the equipment in the laboratory.

### 3.2. Background Science

#### 3.2.1. Phase Transformation and Nucleation

Definite molecular interactions are observed in CASs where the two components are in an equimolar ratio, thus demonstrating higher physical stability (36, 142). One of the major challenges in amorphous materials is that they are prone to recrystallisation. Plenty of co-amorphous systems of BCS class II drugs have been prepared, with solvent evaporation being a popular choice for preparing co-amorphous formulations (10, 184-186). Although it is already established that moisture disrupts the secondary bonding interaction between the components of a co-amorphous system there is not enough research on the same co-amorphous system prepared by different preparation techniques, and how the preparation technique influences stability against recrystallisation and overall performance (10). Turek *et al* compared the performance of a model co-amorphous system of valsartan and nicotinamide prepared by different processing methods but had not tested the stability against recrystallisation by subjecting them to high humidity (10).

The physical stability of amorphous CASs is dictated by crystallisation, which arises from nucleation. Nucleation is the primary step in the formation of a crystal, whether it is from a solution or a solid mixture, i.e., the primary step of a first order phase transformation. A phase is a portion of a system whose properties and composition are uniform and distinct from the rest of the system (187). Phase transformations from one state to the other occur when an initial state is unstable with respect to the final state, and

it is driven by the Gibbs free energy,  $G$  in Equation 3.1, where  $H$  is the enthalpy,  $T$  is the absolute temperature and  $S$  is the entropy of the system. Entropy is the measure of the system's randomness or disorder. Enthalpy is the total heat content of the system and is given by Equation 3.2, where  $E$  is the internal energy, (total of kinetic and potential energy),  $P$  and  $V$  are the pressure and volume of the system, respectively.

**Equation 3.1:** Gibbs Free Energy,  $G$ .

$$G = H - TS$$

**Equation 3.2:** Equation for enthalpy,  $H$ .

$$H = E + PV$$

A system is said to be in equilibrium when it has no energetic reason to transform. A system of fixed mass and composition will stay in a state of stable equilibrium at the lowest possible value of Gibbs free energy in Equation 3.3. There are three states of equilibrium: stable, metastable, and unstable. The stable and metastable equilibrium can be illustrated by Figure 3.1, where stable equilibrium exists at configuration A, when the system is in its lowest energy condition; metastable equilibrium subsists at configuration B when activation energy ( $\Delta G$ ) is needed to attain configuration A and energetic stability. Both these states satisfy Equation 3.3, but only configuration A attained stability at the lowest possible value of  $G$  and unstable equilibrium exists when, with no additional energy, a system would move to a metastable or stable state if disturbed (188). Amorphous systems exist in a metastable equilibrium whilst crystalline systems exist in a stable equilibrium as a solid, i.e., at any given time and condition, the amorphous state may transform to its crystalline form. Amorphous systems exist as disordered arrays of atoms or molecules and are often described as a super-cooled liquid or an undercooled melt, as rapid cooling may not provide enough time for the system to arrange itself to a regular ordered structure.

**Equation 3.3:** Stable Equilibrium.

$$dG = 0$$

Nucleation is a term used to describe the first steps for the formation of a new phase from an old phase in a first order phase transformation. The driving force for nucleation can be obtained from the thermodynamic driving force for first order

transitions (melting or solidifying/crystallisation), when a liquid transforms to stable crystalline form of lower free energy, i.e., dominated by kinetics.

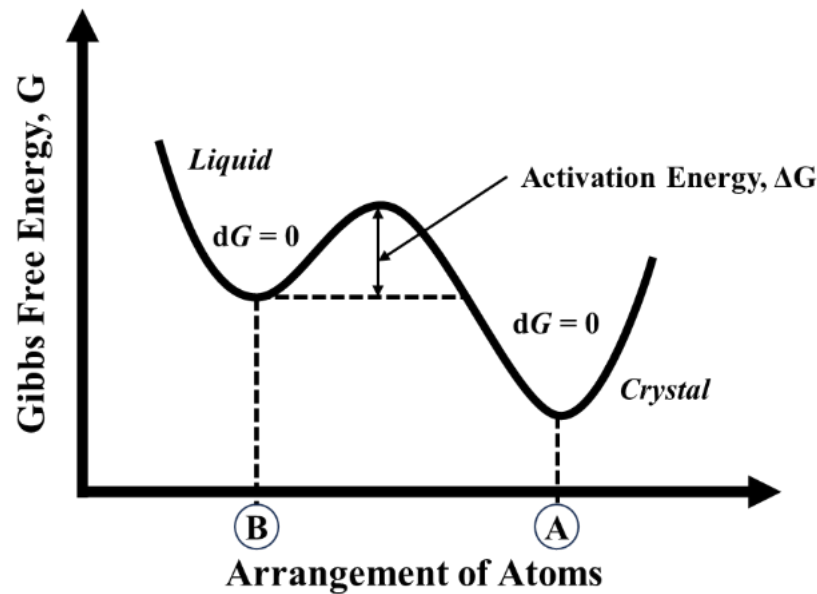


**Figure 3.1:** Schematic variation of Gibbs Free Energy as a function of the arrangements of atoms. A: stable configuration, with lowest free energy B: metastable configuration. (187, 188).

This thermodynamic energy gain is hindered to some extent by the creation of the resultant interface between an old and new phase existing in a mixture (189, 190). Based on the works of Gibbs, Volmer, Weber and Stern, classical nucleation theory (CNT) came into existence. CNT states disordered molecules in a supersaturated solution spontaneously form clusters of crystals by simultaneous variations in density and order till they are big enough to overcome the nucleation barrier, i.e., sufficient number of molecules are required to form a cluster, that has sufficient internal energy to overcome the nucleation barrier, at a critical size.

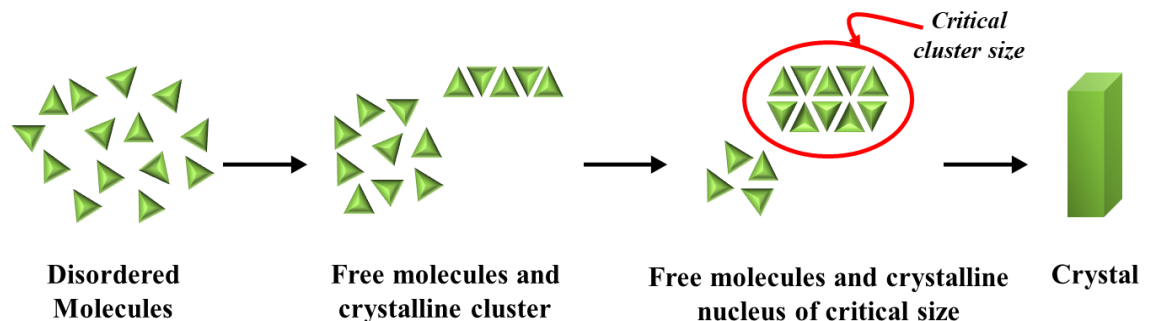


**Figure 3.2:** Schematic of the mechanism of nucleation. Crystalline clusters are formed by attachment of smaller units, till it reaches a critical cluster size, thus forming the crystal nucleus. Beyond that, addition of other molecules leads to the growth of the crystals.

The critical radius ( $r^*$ ) can be defined as the dimension of the nucleus where the internal energy of the cluster is greater than the free energy penalty (surface energy) resulting from the creation of an interface. Clusters of a greater size than the critical radius should be stable and grow to a macroscopic crystal, as seen in [Figure 3.2](#). Nucleation can take place in two ways, namely homogeneous and heterogeneous. Homogeneous nucleation or spontaneous, self-nucleation occurs in the absence of foreign particles or crystals in a solution, and heterogeneous, involves crystallisation on foreign objects in the solution. Both of these are referred to as primary nucleation.

### 3.2.1.1. Homogeneous Nucleation

The random movement of molecules of a liquid with volume  $v$  is dictated by the temperature ( $\Delta T$ ) it is stored at and will sometimes form a solid cluster. At temperatures above the melting temperature of the system ( $T_m$ ) a cluster will decay spontaneously. When  $\Delta T$  is the same as  $T_m$ , the free energy of the liquid is same as that of the cluster. As the liquid is undercooled below the melting temperature, i.e.,  $\Delta T < T_m$  (undercooling), the free energy of the solid falls below that of the liquid. Hence, the creation of a solid cluster of sufficient size suggests a gain in energy by the transformation of liquid to solid. It needs to be of sufficient size because, the formation of a cluster/nucleus consumes some energy to form the interface between the two phases.

The change in free energy due to the formation of cluster of radius  $r$ , by assuming the cluster to be spherical, is given in [Equation 3.4](#) and illustrated in [Figure 3.3](#) and it is the sum of volume free energy (VFE) ([Equation 3.5](#)) and interfacial energy (IE) ([Equation 3.6](#)), where  $\gamma$  is the interfacial energy,  $v$  is volume and  $\Delta G_v$  is the Gibbs free energy change (GFE) per unit volume as a function of undercooling ( $\Delta T$ ) and latent heat of fusion ( $L_v$ ) ([Equation 3.7](#)).

**Equation 3.4:** Change in Gibbs Free Energy associated with the formation of a spherical nucleus of radius  $r$  during a phase transformation.

$$\Delta G_r = -\frac{4}{3}\pi r^3 \Delta G_v + 4\pi r^2 \gamma$$

**Equation 3.5:** Volume Free Energy (VFE).

$$-\frac{4}{3}\pi r^3 \Delta G_v$$

**Equation 3.6:** Interfacial Energy (IE).

$$4\pi r^2\gamma$$

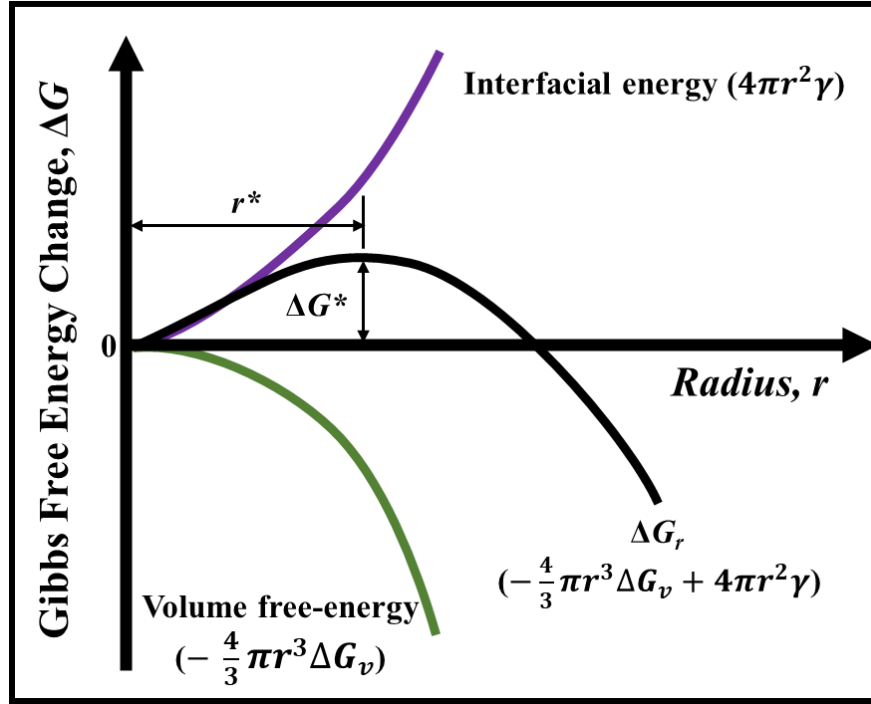


**Figure 3.3:** Free energy during the formation of a cluster as a function of the cluster radius  $r$ .  $G^*$  is the activation energy for the formation of a nucleus of critical size  $r^*$ .

**Equation 3.7:** Gibbs Free Energy change per unit volume for an undercooling  $\Delta T$ , (GFE).

$$\Delta G_v = \frac{L_v \Delta T}{T_m}$$

Figure 3.3 shows that  $\Delta G$  has a maximum height  $\Delta G^*$  at a critical radius  $r^*$ , that acts as an activation barrier against crystallisation. As IE increases by a factor of  $r^2$  and VFE increases by a factor of  $r^3$ , the formation of new solids bigger than  $r^*$  always enhances free energy of the system, but below  $r^*$ , this increase in energy stabilises the metastability at a temperature below  $T_m$  (187). A cluster with a radius smaller than  $r^*$  (embryo) would be thermodynamically unstable and dissipate as dissolution diminishes its free energy. On the other hand, a cluster with a radius bigger than  $r^*$  (nucleus) can lower the free energy by growing in size. By differentiating Equation 3.4, the critical radius  $r^*$  can be obtained, as shown in Equation 3.8 (191).

**Equation 3.8:** The critical nucleus size,  $r^*$ .

$$r^* = \frac{2\gamma}{\Delta G_v}$$

### 3.2.1.2. Heterogeneous Nucleation

In homogeneous nucleation, the activation energy,  $\Delta G^*$  required to form a critical nucleus relies on the IE and GFE, i.e., entirely dependent on the thermodynamic functionalities of the system, thus making it an intrinsic process. Heterogeneous nucleation is an extrinsic process where foreign phases such as walls of the container or embedded particles can contribute to nucleation.

From Equation 3.4 and Equation 3.6, it can be observed that nucleation can take place more easily if the IE is reduced. For heterogeneous nucleation, it can be done so when the crystal nucleus occurs against a foreign object such as the wall of the container (192). The equilibrium of the interfacial tensions ( $\gamma_{SC}$ ,  $\gamma_{CL}$ ,  $\gamma_{LS}$ ) is given by Equation 3.9, where  $\theta$  is the wetting angle and the indices C, S and L represent the container wall (C), solid crystal (S), and undercooled liquid (L). The total IE balances the embryo that is in the shape of a spherical cap against a flat surface, with a wetting angle  $\theta$ , depicted in Figure 3.4.

**Equation 3.9:** Equilibrium of Interfacial Energy.

$$\gamma_{CL} = \gamma_{SC} + \gamma_{LS} \cos \theta$$

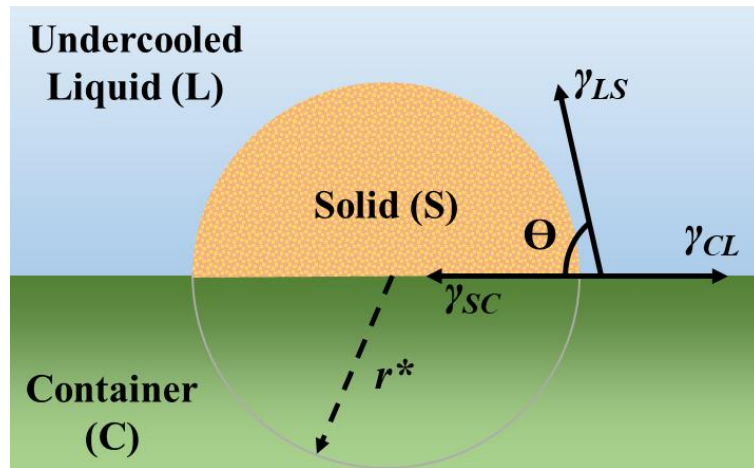


**Figure 3.4:** Heterogeneous nucleation on to a container wall. The nucleus is stabilised through the equilibrium of the interfacial tensions ( $\gamma_{SC}$ ,  $\gamma_{CL}$ ,  $\gamma_{LS}$ ) between container wall (C), solid crystal (S), and undercooled liquid (L) at  $\theta$  is the contact angle.

It is obvious from Figure 3.4 that the volume of a heterogeneous nucleus of critical size  $r^*$  is smaller than that of a homogeneous nucleus of the same critical radius (and decreases with increasing wetting angle) and the difference in volume is given by the shape factor,  $S(\theta)$  in Equation 3.10.

**Equation 3.10:** Shape Factor,  $S(\theta)$ .

$$S(\theta) = \frac{1}{4}(2 + \cos \theta)(1 - \cos \theta)^2$$

$S(\theta)$  is smaller than unity for every contact angle ( $\theta$ ) value less than  $180^\circ$ . Hence the activation energy for nucleation for homogeneous nucleation ( $\Delta G^*_{\text{hom}}$ ) is much higher than that of heterogeneous nucleation ( $\Delta G^*_{\text{het}}$ ) as shown in Equation 3.11 and illustrated in Figure 3.5.

**Equation 3.11:** Relationship between homogeneous and heterogeneous nucleation activation energy.

$$\Delta G^*_{\text{het}} = S(\theta)\Delta G^*_{\text{hom}}$$

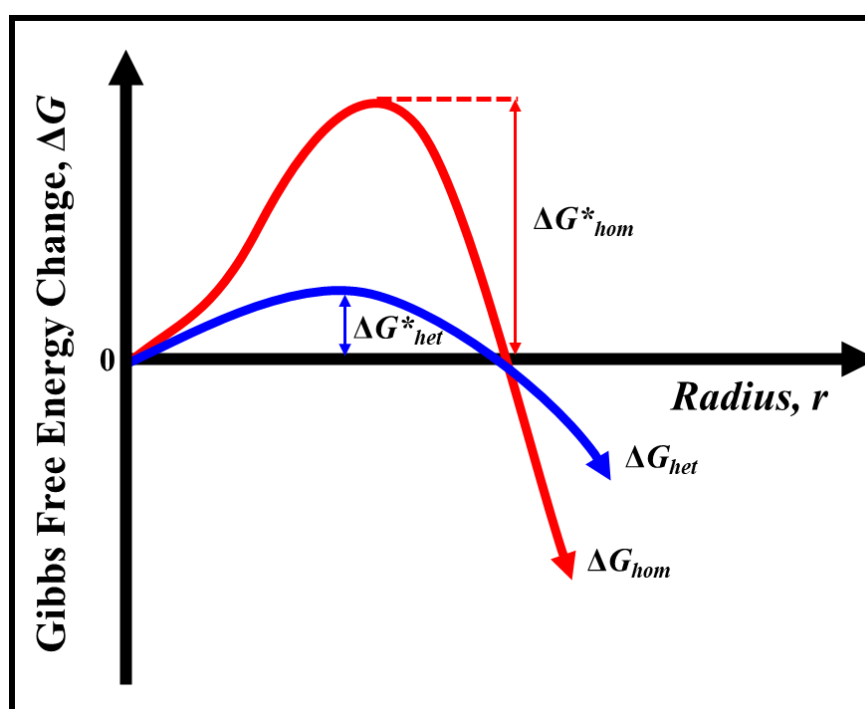


**Figure 3.5:** The excess free energy of solid clusters for homogeneous and heterogeneous nucleation.

The transition from a single-phase homogeneous amorphous system to a heterogeneous system of crystals can occur by either homogeneous or heterogeneous nucleation. As discussed before, heterogeneous nucleation occurs more readily if there are substrates already present in the otherwise amorphous system. In this work, the recrystallisation pathways of the co-amorphous formulation of valsartan and nicotinamide are going to be explored to understand how each of these components undergo phase transformation when they are stabilised by secondary bonding interactions in the formulation and how moisture and time impacts these transformations.

### 3.2.2. Molecular bonding

Molecular bonding is the interaction between molecules to form a stable connection when interacting molecules are in close proximity to each other. It can be classified into primary interactions and secondary interactions.

Primary bonding interactions are strong intramolecular bonds that hold atoms together to form molecules or lattice structures. They can be further classified into covalent bonding (non-metal atoms form molecules by sharing electron pairs), ionic bonding (complete transfer of electrons between metal and non-metal atoms thus forming positive and negative ions that attract each other) and metallic bonding (delocalisation of freely moving valence electrons across a structure of positively charged metal ions).

Secondary interactions are long-range interactions of varying strengths. They can be classified into hydrogen bonds (strong bonding interaction between a hydrogen atom covalently bonded to an electronegative atom and another electronegative atom) and Van der Waals bonding (weak bonding interactions between dipoles).  $\pi$ - $\pi$  interactions resulting from stacking of aromatic rings is a type of van der Waals interaction between the  $\pi$ -electrons of aromatic systems. Both hydrogen bonding and  $\pi$ - $\pi$  stacking interactions can stabilise the disordered state of co-amorphous systems by suppressing phase separation and recrystallisation (137).

### 3.2.3. Amorphisation

Amorphisation is a non-equilibrium phase transition, where a crystalline material loses its long-range order and becomes disarranged with only short-range order or nearest neighbour packing being retained when subjected to thermodynamic or mechanochemical processing.

#### 3.2.3.1. Glass Transition

The glass transition is a second-order phase transition that is not kinetically limited, it is a general characteristic of disordered, supercooled liquids demonstrating a gradual, reversible change in property, where the material transforms from a brittle solid state to a flexible state on heating (193). The glass transition temperature,  $T_g$  is dependent on the heatflow (194).

An amorphous system is a disordered material which lacks long-range order and possesses higher free energy, entropy, enthalpy and mobility compared to its crystalline

counterpart (195, 196). The thermodynamic differences between amorphous and crystalline states are demonstrated in Figure 3.6. Sufficient rapid cooling of a melted crystalline material, below its melt temperature  $T_m$ , does not provide enough time for its molecules to order into the crystalline state, thus freezing in a disordered amorphous state. This supercooled liquid state has higher mobility than a solid but remains viscous in a rubber-like state. Further cooling below the glass transition temperature  $T_g$ , solidifies the material because the diffusion rate of the molecules significantly drops, preserving the disordered state while the material becomes brittle – a characteristic feature of an amorphous phase. It is important to note that upon supercooling a liquid, the difference in entropy of the liquid and solid phase diminishes. Upon extrapolating the heat capacity of the supercooled material below its glass transition temperatures (depending on the cooling rate), that difference in entropies becomes zero and the temperature at which that occurs is known as the Kauzmann temperature ( $T_k$ ).

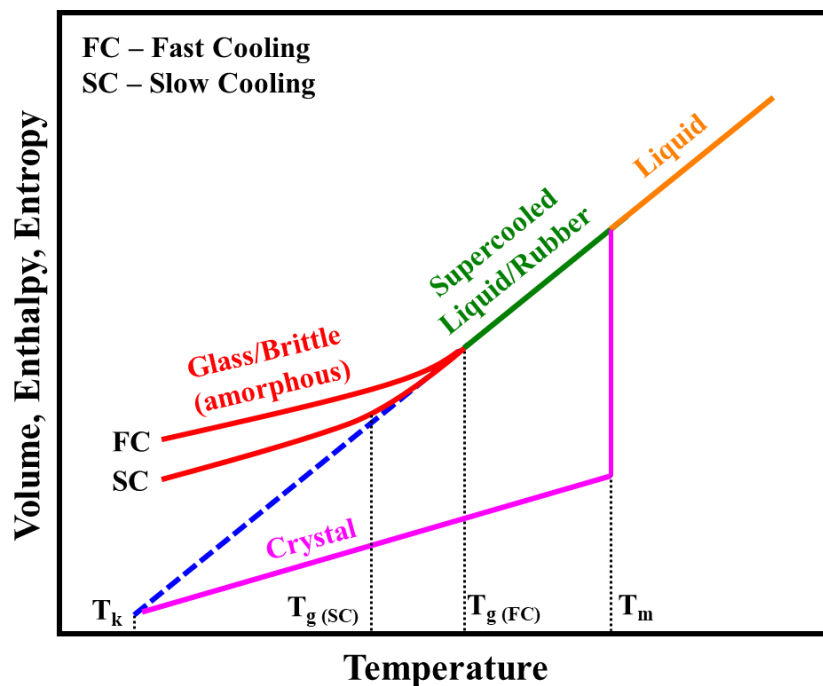


**Figure 3.6:** Thermodynamic properties of crystalline and amorphous states as a function of temperature. The glass transition temperature  $T_g$  varies with cooling/heating rates (194).

The  $T_g$  of a material is the temperature at which the specific heat capacity ( $\Delta C_p$ ) changes during the transition from a glassy to a rubbery state (during heating), located exactly halfway between the extrapolated glassy state and rubbery state baselines, as shown in Figure 3.7. In a single-phase system, a singular glass transition event indicates complete homogeneity. For amorphous mixtures, the theoretical glass transition temperature can be predicted by implementing the Gordon Taylor Equation in Equation

3.12 by using the glass transition temperatures of the starting components and the weight fractions of each (197, 198).

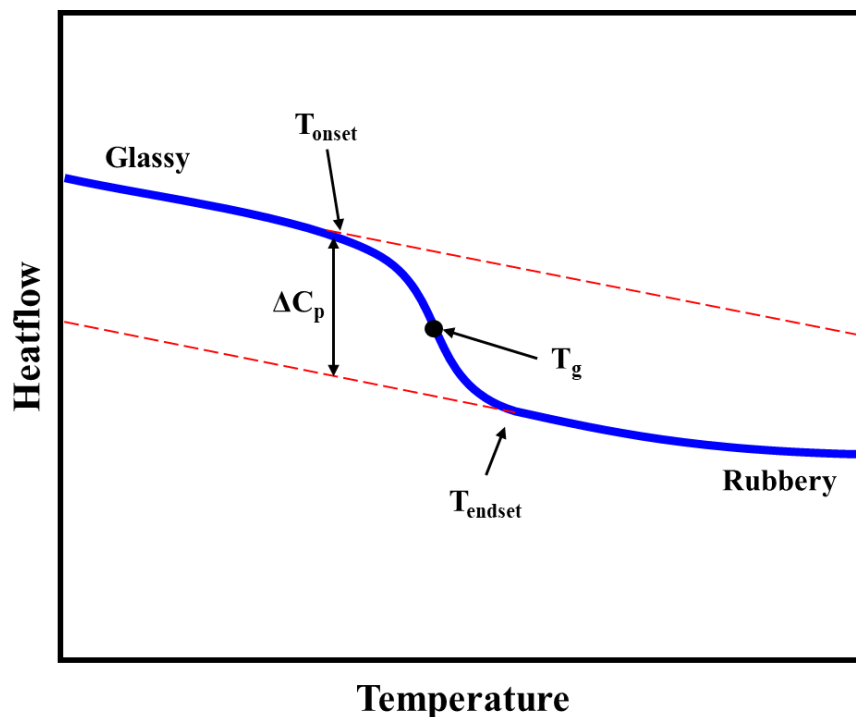


**Figure 3.7:** Schematic representation of a glass transition step in a calorimeter and the  $T_g$  is obtained by using the extrapolated baseline methodology between the glassy state and the rubbery state with the onset and endset ( $T_{onset}$  and  $T_{endset}$ ) temperatures of the transition also marked.

**Equation 3.12:** Gordon Taylor Equation.

$$T_{g1,2} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2}$$

$T_{g1,2}$  is the glass transition temperature of the co-amorphous mixture,  $w_1$ ,  $w_2$ ,  $T_{g1}$ ,  $T_{g2}$  are the weight fractions and the glass transition temperatures for the two amorphous components and  $\rho_1$  and  $\rho_2$  are the densities of each of the components. The constant  $K$  is defined by the Simha-Boyer rule in Equation 3.13 (199).

**Equation 3.13:** Simha-Boyer Rule.

$$K = \frac{T_{g1} \rho_1}{T_{g2} \rho_2}$$

The Gordon Taylor Equation provides researchers with a good approximation of a glass transition temperature between a drug and a co-former in a binary mixture across

its compositional space. Any deviation in real systems is most likely due to a lack of miscibility of the components when loaded in different amounts.

### 3.2.4. Recrystallisation

Recrystallisation is a phase transformation from a disordered high-energy amorphous state to an ordered, low-energy crystalline structure due to heat, time, moisture or all three combined. When an amorphous material is subjected to a temperature above  $T_g$ , the molecular mobility increases which leads to diffusion of molecules that facilitates recrystallisation. Recrystallisation can occur when the amorphous material is stored in a highly humid environment because water molecules can disrupt hydrogen bonding to enable enhanced molecular mobility. Recrystallisation is a first order nucleation and growth process and therefore takes place over time as the molecules cluster in an ordered manner to nucleate a crystal which then grows. This phenomenon can occur by either homogeneous (self-nucleation) or by heterogeneous nucleation on pre-existing particles, crystals, or container walls, depending on the materials storage conditions, mode of preparation or particle size (200).

## 3.3. Materials

### 3.3.1. Valsartan (VAL)

Valsartan ( $C_{24}H_{29}N_5O_3$ ), has a molecular weight of  $435.52 \text{ g mol}^{-1}$  and belongs to the type II angiotensin-receptor blocker (ARB-II) family of drugs which tackle heart failure, diabetic nephropathy, hypertension, thereby reducing the risk of both fatal and non-fatal cardiovascular events such as myocardial infarction (19). VAL has been taken as the API for the co-amorphous system for this project. Valsartan was purchased from Cambridge Bioscience (Cambridge, UK).

### 3.3.2. Nicotinamide (NIC)

Nicotinamide or niacinamide ( $C_6H_6N_2O$ ) is a water-soluble form of vitamin B3, with a molecular weight of  $122.12 \text{ g mol}^{-1}$ , is a nutraceutical with high bioavailability and high aqueous solubility and has been taken as an excipient for this project (29). Nicotinamide was purchased from VWR International (Leicestershire, UK).

### 3.3.3. Solvents and miscellaneous

Ethanol ( $C_2H_5OH$ ) was purchased from Fisher Scientific UK LTD (Leicestershire, UK) as well as supplied by the School of Pharmacy at the University of Copenhagen

during my placement as a research student to prepare co-amorphous systems of VAL and NIC by spray drying.

Measurements of the solubility of the systems were undertaken in water and in phosphate buffer (PB) solution. Milli-Q water was provided by the School of Chemical and Process Engineering at the University of Leeds. PB solution was prepared by mixing approximately 2.1 g of sodium phosphate monobasic monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ) and 10.05 g of sodium phosphate dibasic heptahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ) with molecular weight of  $138 \text{ g mol}^{-1}$  and  $268.07 \text{ g mol}^{-1}$  respectively, in 500 mL of water, to attain a pH of 7.4, which was recorded by a ETI 8100 pH Meter Kit. The PB solutes were purchased from Thermo Fisher Scientific (Waltham, USA) and the pH meter was purchased from Electronic Temperature Instruments Ltd, UK.

Potassium chloride (KCl) was purchased from Alfa Aesar (acquired by Thermo Fisher Scientific, Leicestershire, UK) and silica gel was purchased from Fisher Scientific UK LTD (Leicestershire, UK) for the ageing study conducted at 85% (wet conditions) and 25% RH (dry conditions) respectively.

### 3.4. Preparation Techniques

#### 3.4.1. Melt Amorphisation



**Figure 3.8:** Equipment for melt amorphisation, mortar and pestle to mix the sample and a heating oven, where the sample is melted.

Quench cooling is a rapid cooling process for materials to alter their physical properties or to obtain materials with a desired quality in mind (201). It involves heating a substance above a certain temperature and then rapidly bringing the temperature down i.e., cooling by placing it in an environment of a lower temperature or submerging in a cryogenic medium such as cold water or liquid nitrogen.

Melt amorphisation is an example of quench cooling, where the API is heated at a temperature beyond its melting point and then cooled by placing it in a cooling medium, which prevents the molecules of the API from rearranging back to a crystalline state, thereby leaving them in a disordered, amorphous state, which can provide higher solubility and bioavailability of the drug. Evidence for an amorphous state is the presence of a single endothermic glass transition and the lack of a melting endotherm in the material's DSC data and a lack of crystalline peaks in the pXRD pattern. For this processing technique, mortar, pestle, and heating oven were the only equipment needed for the preparation of the binary amorphous systems, as demonstrated in [Figure 3.8](#). This technique has been implemented in this project to prepare amorphous form of VAL as well as co-amorphous formulations of VAL and NIC. For the preparation of the co-amorphous formulations of VAL and NIC, the two components were accurately weighed out and fully mixed in a mortar and pestle for 5 minutes. The mixture was heated in a SciQuip Oven-30S (Newton, England) up to 150 °C, a temperature beyond the melting points of both the components. After reaching 150 °C, the sample was cooled down to room temperature, by being taken out of the oven, and placed under normal room conditions. It solidified into a transparent solid, which was extracted from the crucible by dragging a metal spatula through it, this resulted in the formation of a loose white powder, as shown in [Figure 3.9](#).

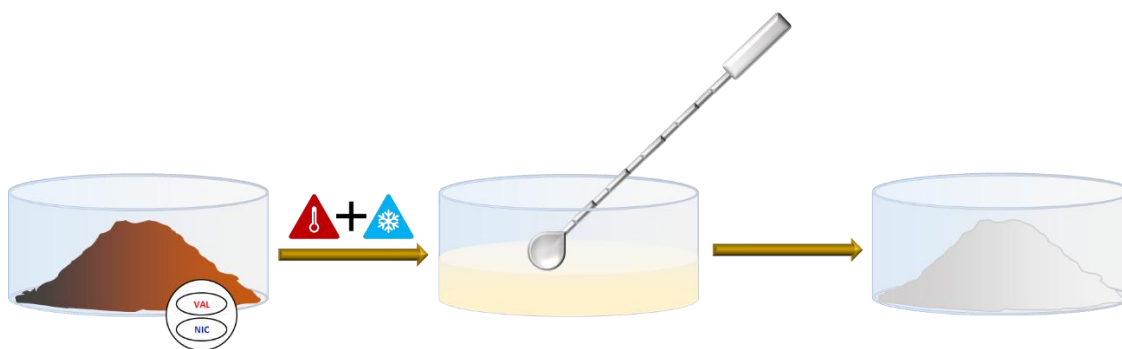


**Figure 3.9:** Schematic of melt amorphisation. The ground up mixture of VAL and NIC are placed in a flat-bottomed beaker, which was heated to 150 °C and cooled at room temperature, transforming into a transparent mass, which further transforms into loose white powder upon extraction stress.

This technique is useful as it is easy to prepare many samples in one go. The swift nature of this preparation technique has been taken advantage in this project, where melt amorphisation has been used to prepare multiple ratios of VAL-NIC CASs to identify the optimal ratio that has the least retained crystallinity as well as presumed stability against recrystallisation. Identification of the optimal ratio has been a key factor in this project as

CASs of VAL and NIC were prepared by different methods by maintaining that ratio and the long-term studies of the stability and performance of those systems under high humidity was investigated.

### 3.4.2. Ball Milling

Ball milling is a mechanochemical technique that reduces the size of materials into fine powder by using a vessel with grinding balls inside it. Although different kinds of ball mills can be used for pharmaceutical research, mixer mills are extremely popular due to their versatility. They are efficient in reducing particle size of APIs thereby enhancing solubility and dissolution rate, which in turn enhances bioavailability and ensures uniformity in the particle size distribution compared to larger mills. This improves homogeneity in the formulation, all of which ultimately ensures consistency in dosage. They can work with different kinds of material, whether its dry, wet or systems that are thermally sensitive. Furthermore, these instruments are Good Manufacturing Practice (GMP) compliant and are easy to use and maintain. Ultimately, these small-scale mixer mills can be the precursor to large-scale industrial production, which is beneficial for ensuring batch-to-batch consistency during the transition to large-scale operation. Ball milling in general has been utilised to prepare amorphous forms of drug delivery system by combining APIs with polymers or smaller molecules, all of which have resulted in improved solubility and dissolution rates (202, 203).

A mixer mill consists of a motor, grinding chamber and control panel as demonstrated in [Figure 3.10](#). The motor lies within the instrument that performs the milling. The grinding jars perform the grinding with grinding balls within it. The jars are either made from stainless steel or zirconium oxide (ceramic) and have volumes of 25 to 50 mL and the grinding balls, like the jars, are either made from stainless steel or zirconium oxide having varying sizes, from 3 mm all the way up to 30 mm). The control panel controls the functioning of the operation by regulating the duration and vibration (shaking) of the operation.

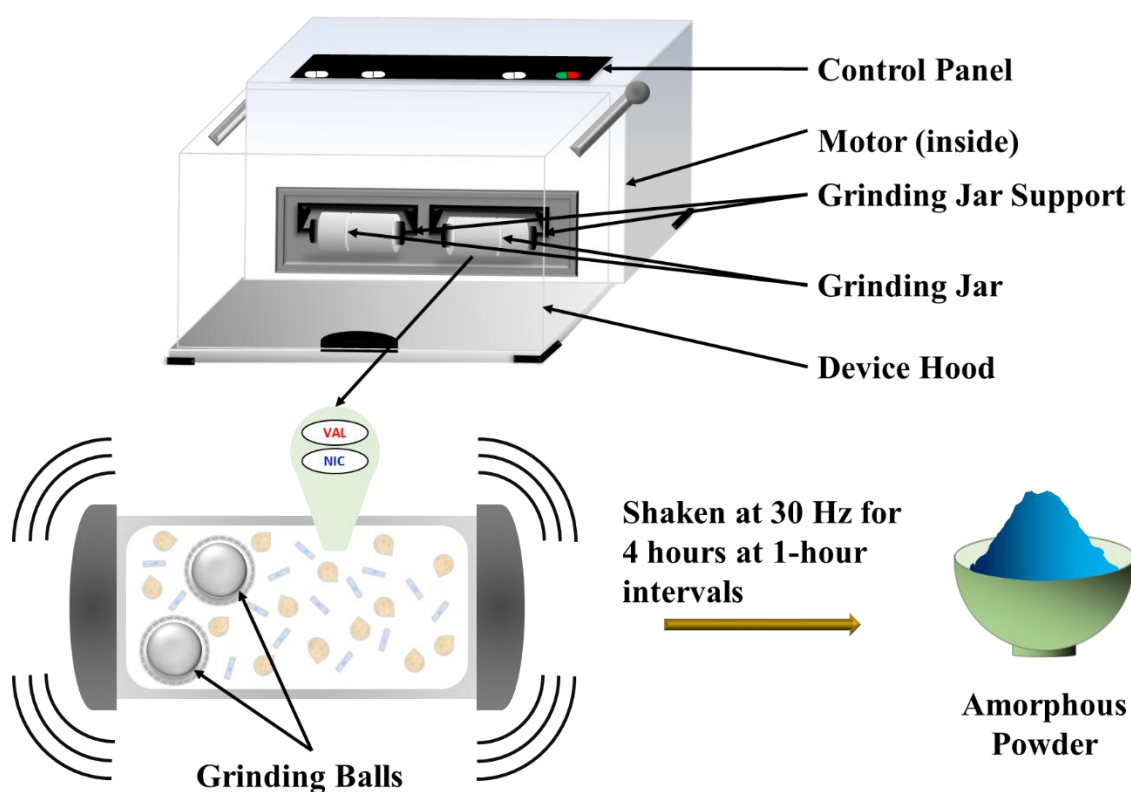


**Figure 3.10:** Schematic of ball milling where equimolar amounts of the starting material (VAL and NIC) were shaken at 30 Hz for 4 hours, every 1 hour to prepare co-amorphous powder.

In this project, equimolar co-amorphous system of VAL and NIC mixtures were prepared by ball milling a total of 1.12 mg powder of the crystalline starting materials for 4 hours, at 1-hour intervals, as depicted in [Figure 3.10](#). The samples were milled in Mixer mill MM400, (Retch GmbH & Co., Hann, Germany) at a frequency of 30 Hz in 25 mL stainless steel grinding jars containing two stainless steel balls with a diameter of 12 mm, placed in a cold environment (6 °C).

### 3.4.3. Slurry Mixing

Slurry mixing is a solvent evaporation technique that involves the formation of a uniform solution of the starting components in a solvent under heat, which is then subjected to rotary evaporation to obtain a dried product. Rotary evaporation eliminates volatile solvent from a sample under pressure, rotation, and controlled heat, all three of which can be selected by the user in accordance with experimental requirements.

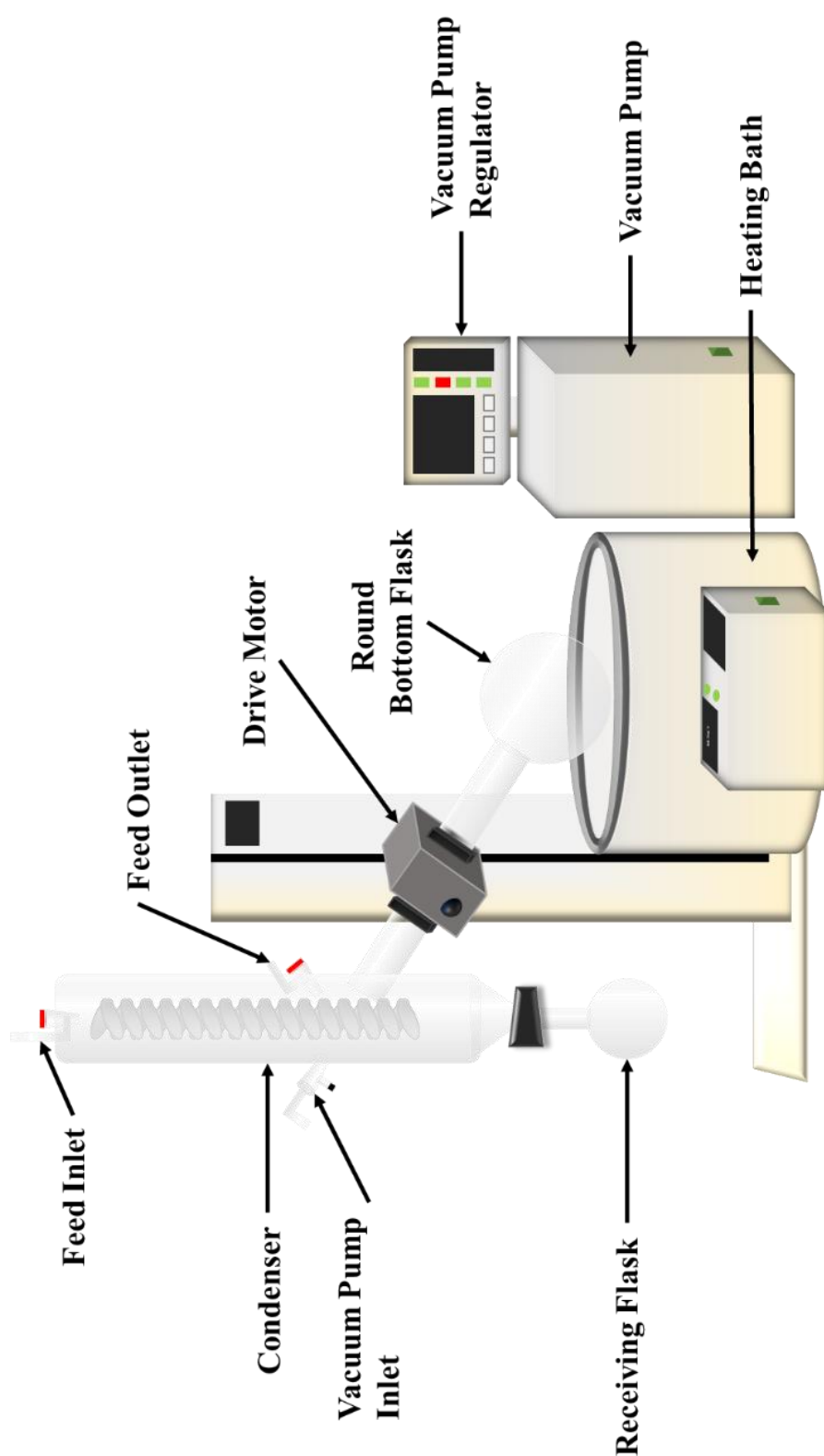


**Figure 3.11:** Schematic of a rotary evaporator.

A rotary evaporator is composed of an evaporation container, a heating bath, a rotary motor, a condenser, a vacuum system and receiving flask as shown in [Figure 3.11](#). An evaporation container is usually a round bottom flask that holds the solution that is up for drying and is attached to the drive or rotary motor. The rotary motor spins the round

bottom flask at a speed set by the user, which increases the surface area of a sample and aids in quicker evaporation. The heating bath is where the flask rotates, submerged in either oil or water, which is heated at a constant temperature. A condenser is a coiled tube within a glass body which condenses the solvent vapours into liquid by circulating a cooling liquid through the coils. The cooling liquid enters the condenser through the feed inlet and outlet attached to the coils and the glass body is connected to the rotating round bottom flask and the receiving flask. A vacuum pump induces vacuum into the evaporation operation which reduces the pressure within the system thereby lowering the boiling point and speeds up evaporation. The receiving flask collects the condensed liquid solvent which has been separated from the solution in the round bottom flask.

Such a solvent evaporation technique can be used to prepare co-amorphous system by dissolving the components in a solvent, in the presence or absence of heat and then subjecting the solution to heat and vacuum to facilitate evaporation, which transforms the material into a non-crystalline, amorphous state. It is relatively an inexpensive operation, but the time taken to prepare the amorphous material may vary. The resulting product may be metastable, making it prone to recrystallisation. To mitigate instability, factors like temperature, vacuum, rotation speed, and solids load must be carefully optimised. The resulting amorphous materials prepared by rotary evaporation have higher solubility and dissolution rates than their crystalline counterparts (204-206).

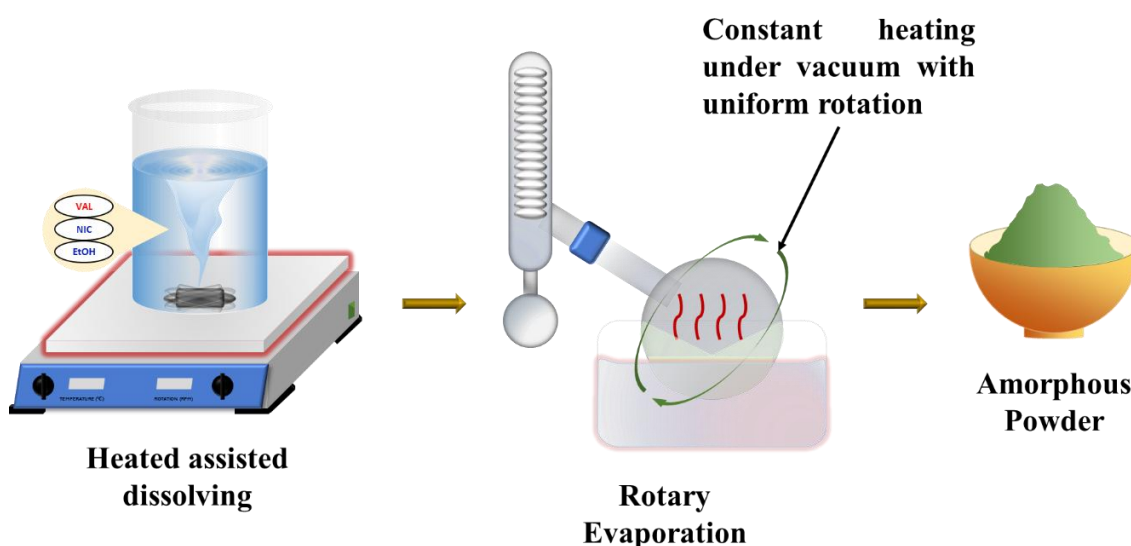


**Figure 3.12:** Schematic of heated slurry. VAL and NIC dissolved in heated ethanol is subjected to rotary evaporation at 60 °C at 10 mBar for approximately 6-8 hours to obtain dry amorphous powder.

In this project, equimolar amounts of VAL and NIC were heated in boiling ethanol for approximately 6 minutes. Rotavapor R-100, in combination with Heating Bath B-100,

Vacuum Pump V-100 and Interface I-100 (Büchi, Flawil, Switzerland) and an external temperature-controlled heat circulating bath (PolyScience, Illinois, USA) were used to prepare this co-amorphous system. The system was dried under vacuum at 10 mBar, in a round bottom flask in a water bath at 60 °C for approximately 6-8 hours. After the solvent has been evaporated, the flask is removed, and the dried powder is obtained as demonstrated in [Figure 3.12](#).

#### 3.4.4. Spray Drying

Spray drying is a thermodynamic, solvent evaporating process of producing dry powder from a solution by rapidly drying a liquid mixture with hot air. It works by atomising the solution to disperse the liquid and by supplying heat to the system to dry the moist air and collect the dry powder at the end.

A spray dryer is composed of a feed system, an atomiser, a heating system, a drying chamber, a collection system, an air system, and operating panel for the operation as shown in [Figure 3.13](#). The operating panel exhibits all the functions to control the spray parameters plus the inlet and outlet temperature. The feed system consists of a feed inlet, a peristaltic pump and the vessel carrying the liquid feed to transfer the solution into the atomiser at a controlled rate. The atomiser transforms the liquid feed into a fine mist of tiny droplets (atomisation) into the drying chamber. In many spray dryers, the use of two-fluid spray nozzles as atomisers are common, which transmits the drying air with the feed stock to atomise the liquid. Compressed air is used in the operation of spray drying, providing the necessary pressure for atomising the feed. The heating system includes the inlet and outlet temperature, where the former heats the drying gas to remove moisture from the atomised feed, thereby controlling the drying rate while the latter monitors the dryness of the final product. The drying chamber is where the atomised liquid feed comes in contact with the hot drying gas where the liquid evaporates rapidly. The collection system consists of a cyclone that separates the dried particles, a product collection chamber where the dried sample accumulates after the drying process and a filter that collects any remaining particles that may be present in the moist air.

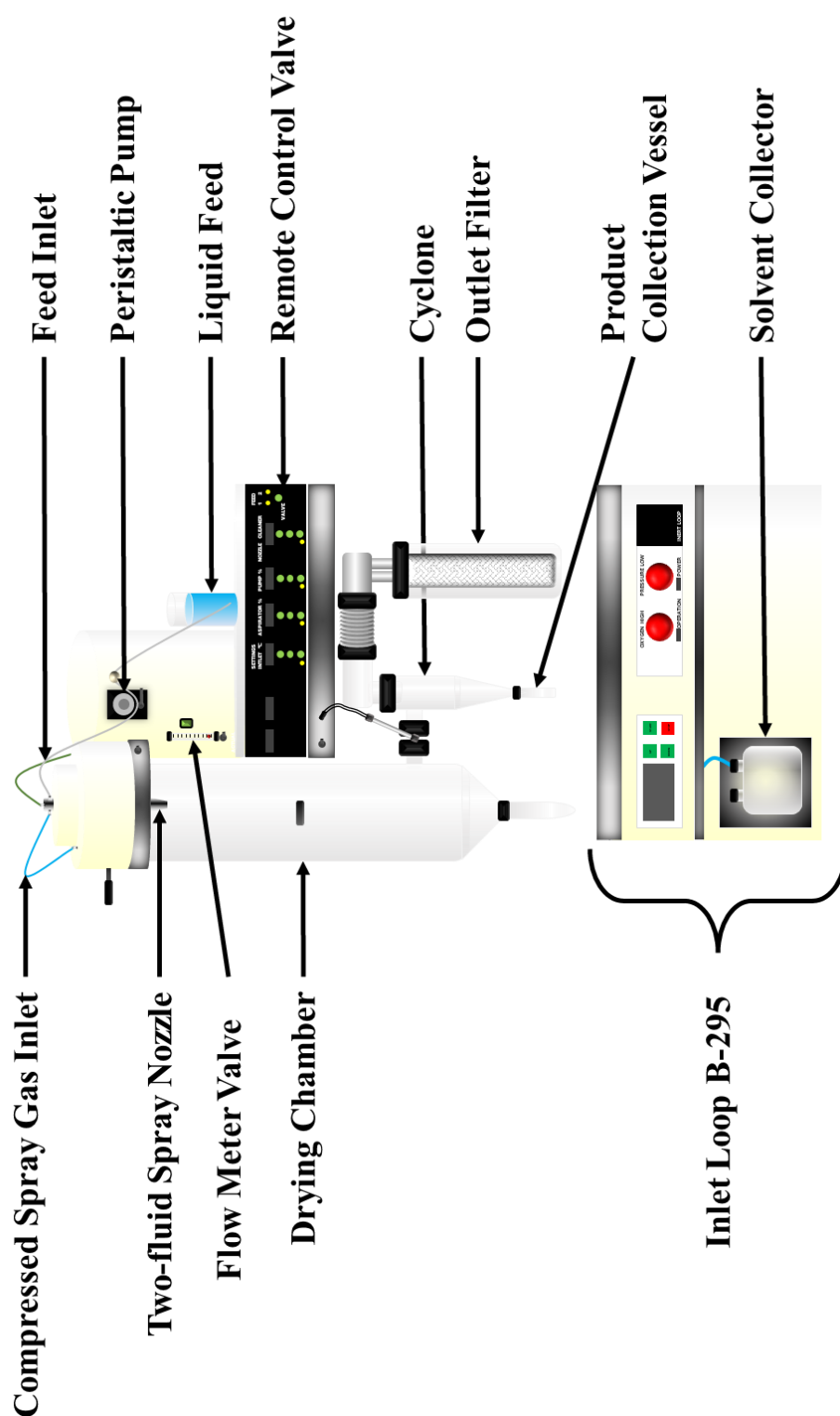


**Figure 3.13:** Schematic of a spray dryer.

The air system is composed of an air distributor that transmits compressed air into the drying chamber and a cooling block that is connected to the spray dryer (such as the inert loop B-295 connected to Buchi spray dryer B-290). It ensures the safety of the spray drying when using flammable solvents. This closed loop circulation operates under inert conditions where the circuit automatically shuts down if the oxygen level is too high to

prevent explosion. This also prevents the start of an operation if the oxygen levels are too high. Nitrogen is used to displace the oxygen. With the displacement of oxygen, the temperature within the drying chamber is also reduced, thereby ensuring further safety. The atomisation of liquid feed to collection of dry sample powder has been demonstrated in [Figure 3.14](#).

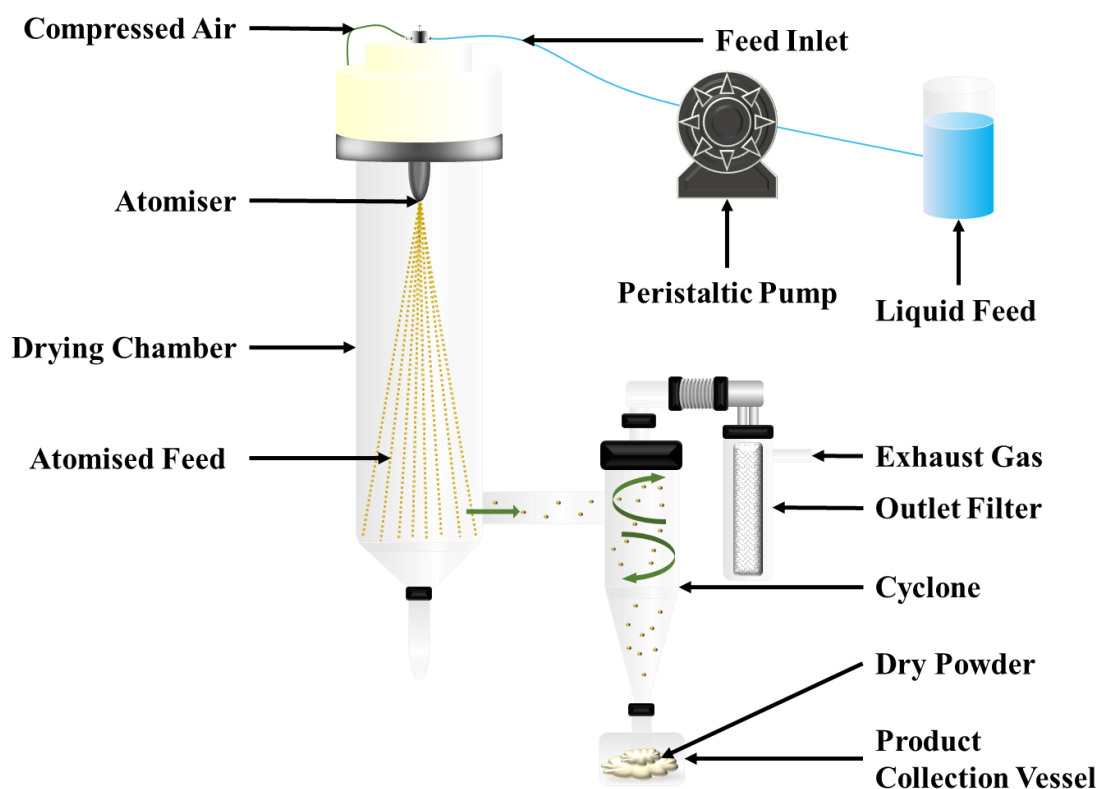


**Figure 3.14:** Schematic of spray drying. The liquid feed is atomised by the atomiser which is then heated in the drying chamber by hot air, regulated by the operating panel and then is collected in the collection vessel as a fine drying powder. The filter eliminates excess particles that may be present in the moist air before it is expelled from the drying system.

Spray drying has a multitude of applications in many industries such as the chemical industry, the food and beverages industry and the pharmaceutical industry (207). In pharmaceutical research, stable amorphous form drug delivery systems can be prepared by combining APIs with polymers or small molecules like amino acids or even other APIs, and these have been shown to enhanced solubility and dissolution rate (75, 161, 208). Such a preparation technique provides control over the particle size and morphology to influence the performance i.e. solubility and bioavailability of the drug delivery systems. Powders with least amount of moisture have the most stability of the metastable amorphous form products against recrystallisation. Moisture facilitates recrystallisation as well as encourage microbial growth (184, 209). It is a useful technique

to work with when using thermally sensitive compounds and prevent them from degrading during processing. Overall, spray drying is a cost-effective, scalable continuous processing technique that can produce powders for a wide range of products for oral and inhalation drug delivery systems, in a single continuous step (210).

In this project, co-amorphous systems of VAL and NIC was prepared by spray-drying with a Mini Spray Dryer B-290, equipped with the Inert Loop B-295, a high-performance cyclone (Büchi, Flawil, Switzerland) and an external Ismatec Reglo Analog peristaltic pump (IDEX Health and Science, Glattbrugg, Germany). Compressed air was used as the atomising gas. For each spray-drying run, 3.35 g of equimolar mixture of VAL and NIC were dissolved in 1000 mL of 50/50 (v/v) ethanol/water solvent mixture. During all runs, the atomising gas feed and aspirator rate were kept constant at their maximum levels of 100%, respectively. Nitrogen was used with the Inert Loop to displace oxygen thereby preventing the generation of an explosive mixture when drying the flammable solvents.

#### 3.4.5. Test of stability: Ageing Study

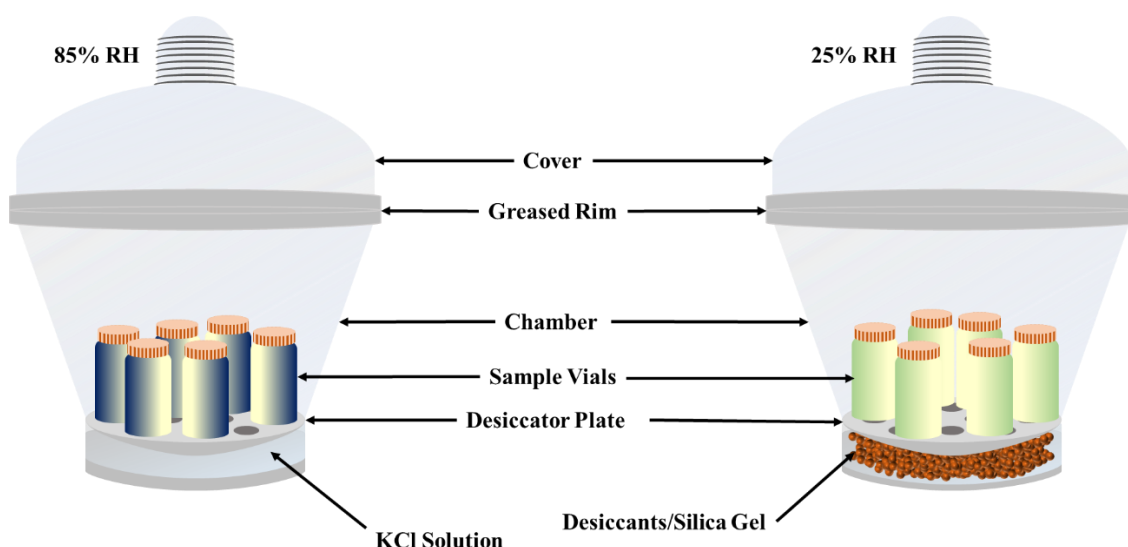


**Figure 3.15:** Test of ageing by storing samples at different humidities of 85% and 25% RH.

The binary co-amorphous systems were tested against two different relative humidities (RHs) of 85% and 25% as demonstrated in Figure 3.15. The humidity chamber with 85% RH was prepared by keeping a supersaturated salt solution of potassium chloride (KCl) inside a desiccator. The systems were also placed in a desiccator with silica gel within which the relative humidity was 25%. The two different RHs were measured using hygrometers. The ageing study has been carried out until recrystallisation

was observed; the study was stopped as soon as all the co-amorphous systems displayed crystallinity by pXRD. Hygrometers (Hygrometer Testo 608-H2) used for the ageing study to keep a track of the relative humidities were purchased from Onecall (Premier Farnell, Leeds, UK).

### 3.5. Characterisation Techniques

#### 3.5.1. Powder X-Ray Diffraction (pXRD)

Powder X-ray Diffraction (pXRD) is the most common, non-destructive analytical technique used in the characterisation of pharmaceutical materials to identify phases, determine crystal structures, and quantify sample composition. In the pharmaceutical industry, pXRD is crucial in identifying the solid-state characteristics of the drug delivery system, be it crystalline, amorphous, or polymorphic. Such a technique ensures effective quality control, from the early stages of research of the development of the product to the finalised product awaiting regulatory approval.

An X-ray instrument consists of three main items: an X-ray source, a sample stage, and an X-ray detector, as depicted in Figure 3.16. For a pXRD analysis, the sample is ground finely to guarantee a random orientation of crystalline particles and loaded and pressed onto a flat holder.

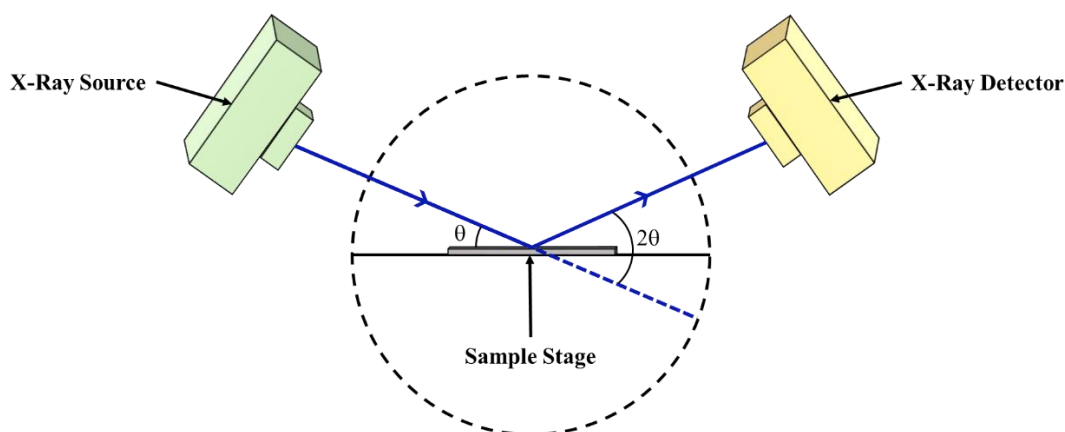


**Figure 3.16:** Schematic of an X-ray diffractometer.

X-rays are a form of high-energy electromagnetic waves with wavelength that extends from 0.01 nm to 10 nm. They are produced by accelerating electrons from a heated filament at a metal target (usually copper) which knock atomic electrons out of the K-shell orbitals, leaving them at a high energy state. An electron from a higher energy level, likely L or M shell, will then descend to fill the vacancies of the inner shells of the

target atom thereby releasing energy in the form of X-rays. This creates a series of  $K_{\alpha}$  (from L-shell) or  $K_{\beta}$  (from M-shell) X-rays when atomic electrons fall from the higher shells. The L-shell is made up of three sub energy levels (one s orbital and two p orbitals), with the radiation only emerging from the p orbitals as in the form of two wavelengths,  $K_{\alpha 1}$  ( $\lambda = 1.5406 \text{ \AA}$ ) and  $K_{\alpha 2}$  ( $\lambda = 1.5443 \text{ \AA}$ ), with the latter removed by the computer software to produce clear data with better resolution.

The X-rays produced by the source interact with the periodic crystal lattice of the sample, constructively interfere at specific angles when the path difference of rays from consecutive crystal planes is an integer number of wavelengths, which upon reflecting creates distinctive diffraction patterns on a detector, observed as a function of diffraction angle ( $2\theta$ ). The pXRD patterns can be analysed for the identification and quantification of the diffraction peaks, by measuring their positions and intensities, and matching the pXRD data to known crystal structures present in databases such as the International Centre for Diffraction Data (ICDD) or Cambridge Structural Database (CSD).

**Equation 3.14:** Bragg's Law

$$n\lambda = 2d\sin\theta$$

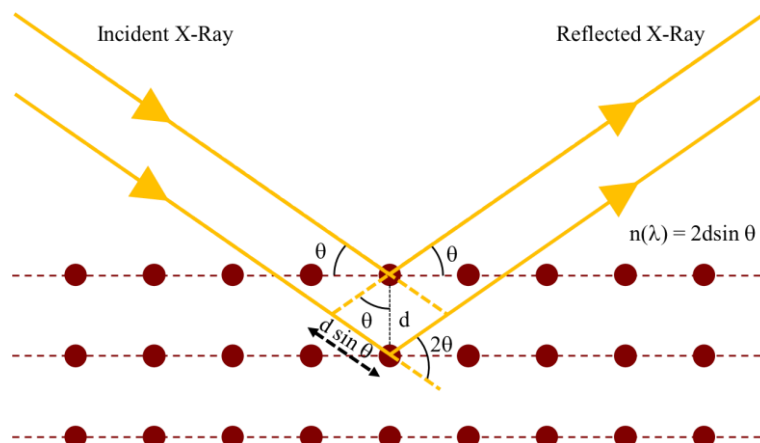


**Figure 3.17:** Geometric representation of Bragg's Law demonstrating the diffraction of X-rays from lattice plane with  $d$  as the interplanar spacing within the crystal lattice and  $\theta$  as the angle of incidence and reflectance.

The fundamental principles of pXRD are described by Bragg's Law. The diffraction angle ( $\theta$ ) is related to the wavelength ( $\lambda$ ) of X-rays and the interplanar spacing ( $d$ ) within the crystal lattice by Bragg's Law, which states that an incident X-ray onto a crystal surface with its angle of incidence,  $\theta$ , would reflect with the same angle of scattering,  $\theta$  and when the interplanar spacing  $d$  is equal to a whole number  $n$  of the

wavelength  $\lambda$ , constructive interference will occur, and it is presented in [Equation 3.14](#) and [Error! Reference source not found.](#)

In this project, pXRD has been implemented by a Malvern Panalytical Aeris diffractometer (research edition, Malvern Panalytical, Malvern, UK) to determine the structure of the co-amorphous systems of valsartan and nicotinamide prepared by four different techniques as well as study the bulk crystallinity of these systems when stored at different humidities for 32 weeks. The X-rays were generated by a copper long-fine focus tube ( $\lambda = 1.5406 \text{ \AA}$ ), operating at 40 kV and 7.5 mA. Each sample was loaded on to a metal holder, with a silica cup and analysed using the diffractometer in a  $\theta$ -2 $\theta$  configuration over a 2 $\theta$  scan range of 5° to 50°, step size of 0.0217° and step time of 198.645 seconds, and a divergence slit of 1/4° which reduces background noise and diffused X-ray scattering. Upon reducing such background, the resolution of the diffraction pattern is better for analysis. Data analysis was performed on the XRDMP Creator 1.3.0.0 and Highscore Plus software in the form of peak profile fitting and by comparison to data in the International Centre for Diffraction Data (ICDD) database and Cambridge Structural Database (CSD).

### 3.5.2. Thermal Analysis (TA)

Thermal techniques are implemented heavily in pharmaceutical analysis (142, 149, 211, 212). They characterise a system by observing the changes in the physiochemical properties of a compound or a blend of compounds as a function of increasing temperature. The two primary techniques of such thermal analysis are thermogravimetric analysis and differential scanning calorimetry, both of which are explained in the next sections.

#### 3.5.2.1. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal analysis technique which monitors the mass loss of a material over time against temperature changes at a constant heating rate, which assists in determining its chemical composition and predicting its thermal stability. This analysis technique provides information about the sample's physical characteristics such as gas adsorption, phase transitions and chemical characteristics such as thermal decomposition (213). Thermogravimetric analysis can broadly be classified into three categories:

- Isothermal or static thermogravimetry: the mass of the sample is monitored as a function of time at a constant temperature during the entire analysis
- Quasistatic thermogravimetry: the sample is heated in a series of sequential steps separated by isothermal intervals where the sample is held at a constant temperature till the sample mass reaches stability before the beginning of the next temperature ramp
- Dynamic thermogravimetry: the sample is heated at a constant, linear rate, and the change in mass of the sample is recorded as a function of temperature (continuous heating)

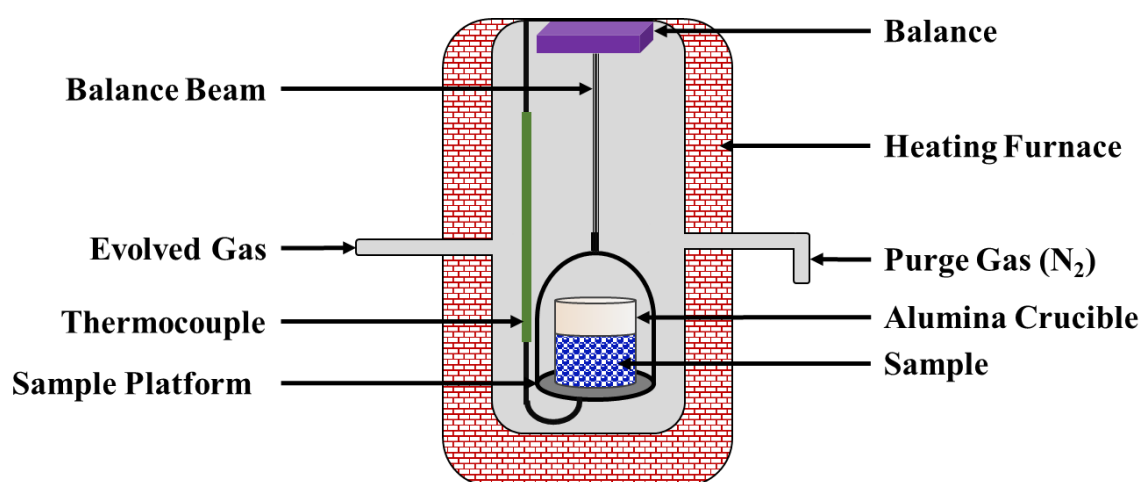


**Figure 3.18:** Schematic of a TGA instrument.

The key components of a TGA instrument are a high-precision balance to measure the sample's mass, a furnace for controlled heating, sample platform, thermocouple to monitor temperature changes and a purge gas system, typically nitrogen gas, as depicted in [Figure 3.18](#). The balance in a TGA is highly sensitive, with the sensitivity levels of the instrument are around 0.1  $\mu\text{g}$ , and it accurately measures the mass of the sample as a function of temperature and time. The sample platform is where the crucible with the sample is loaded and unloaded. The crucible should be filled halfway, ensuring the bottom of the crucible is covered with the sample. It is gently then loaded onto the sample platform, and the balance records the mass of the sample, provided the mass of the crucible has already been tared to zero. For this project, a Shimadzu TGA-50 instrument (Shimadzu Corporation, Kyoto, Japan) was implemented to conduct TGA, under a N<sub>2</sub> atmosphere at a flow rate of 50 mL min<sup>-1</sup>. About 5 to 20 mg of powder was loaded to an alumina crucible (can be heated to over 1600 °C) and heated at a temperature ramp rate of 10 °C min<sup>-1</sup>, from 25 to 600 °C. Mass loss was recorded as a function of temperature.

The results of a TGA measurement are displayed as a TGA curve in which the mass loss or percentage of mass loss are plotted against temperature or time or both.

TGA is beneficial to pharmaceutical research and development by identifying the optimal storage conditions to prevent premature degradation of drug systems. It is important for obtaining information about the moisture content within these systems, which could affect their stability by disrupting their physical structure or cause moisture-induced degradation. In this project, dynamic TGA has been implemented for observing moisture adsorption by the co-amorphous systems as well as monitoring the mass loss changes with varying ratios of starting components. Furthermore, it has been useful for determining the temperature at which thermal degradation commences for the pure components as well as the binary systems.

#### **3.5.2.2. Differential Scanning Calorimetry (conventional and temperature modulated) (DSC and tmDSC)**

Differential scanning calorimetry (DSC) is another widely used thermal technique, which is quick to provide an analyst with a wealth of information about the material they are analysing. It is a powerful analytical technique that is extensively used by different industries, from the food industry to infrastructure industry (214). In the pharmaceutical industry, it is used to characterise APIs, excipients, and DDSs. DSC measures the heat flow associated with temperature changes in a sample during heating, cooling, or remaining isothermal, offering insights into phase/structural changes, chemical reactions, and physical interactions. These measurements give the analyst quantitative and qualitative information regarding the physicochemical changes in the sample that involve endothermic or exothermic processes and variations in heat capacity.

In conventional DSC, the difference in heatflow between a sample and a reference is measured as a function of time and temperature given that both the sample and reference are subjected to the same controlled environment of time, temperature, and pressure.

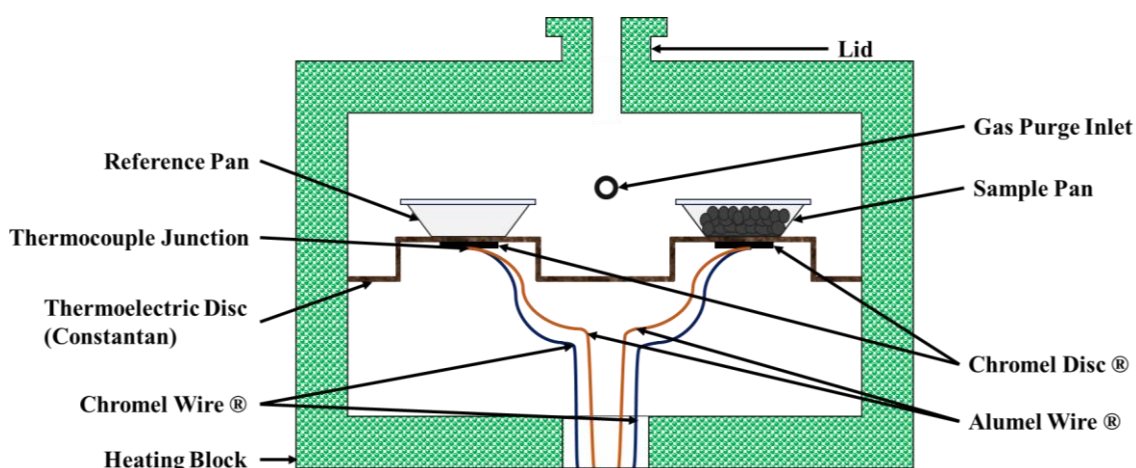


**Figure 3.19:** Schematic of Heat flux DSC. Chromel ® and Alumel ® are registered trademarks of Hoskins Manufacturing Company (215).

A DSC instrument is made up of a heating block or the furnace, hermetically sealed sample and reference pans, thermocouples and their wiring and a purge gas system, as depicted in [Figure 3.19](#). The sample pan is where the sample is placed for thermal analysis, and the reference pan is required to provide a predictable baseline for heatflow in and out of the pan. These pans are identical in shape, volume, weight, and material and are typically made of aluminium. By having a reference pan, the instrument can identify and record the difference between the heatflow of the pan and the sample, thus generating a specific thermogram of the sample and not a combination of the sample and the pan. The lid of the sample pan is often punctured with a hole to prevent the building up of pressure in the system; aluminium pans would deform under pressure, which could lead to the sample leaking out and affecting the surface within the heating block and create potential artefacts in the data recorded. The sample needs to be in good thermal contact with the pan, i.e., the sample must cover the base of the pan entirely. The purge gas, such as nitrogen, is used to control the sample environment by purging volatiles emitted from the sample, thereby preventing contamination. A thermoelectric disk made up of constantan alloy (in the instruments used for tmDSC in this project) transfers heat to and from the pans. The heat transferred through the disk is recorded by type K thermocouples at the junction of the Chromel ® disks and thermoelectric disk. Such a thermocouple can record temperatures between -200 °C to +1260°C. The wires, Chromel ® and Alumel ® attached to the disks measure sample temperature directly. The differential heatflow is measured by the thermocouples by using the Ohm's Law in [Equation 3.15](#).

**Equation 3.15:** Thermal Ohm's Law.

$$\frac{dQ}{dt} = \frac{\Delta T}{R_D}$$

Where  $\frac{dQ}{dt}$  is the heatflow,  $\Delta T$  is the difference in temperature between the sample and the reference and  $R_D$  is the thermal resistance of the thermocouple disk. Chromel ® is a 90% nickel – 10% chromium alloy used as the positive wire in a Type K thermocouple whereas Alumel ® is an alloy which is approximately 95% nickel with low quantities of aluminium, magnesium, and silicon, acting as the negative wire of a Type K thermocouple.

A typical DSC experiment generates a heat flux curve as a function of temperature. Figure 3.20 shows that first-order transitions, such as melting and crystallisation, appear as peaks, while second-order transitions, such as glass transitions, appear as a shift in the baseline of the thermograms. Peak directions are positive and negative, corresponding to exothermic and endothermic reactions respectively and occurring within the sample during the experiment.

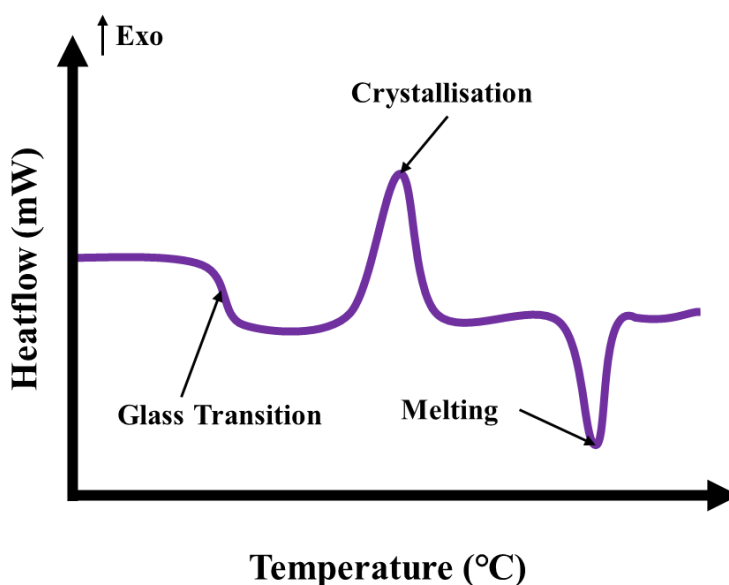


**Figure 3.20:** A typical DSC thermogram with common identifiers.

Although conventional DSC has many advantages such as having a fast analysis time, easy sample preparation regardless of the physical state of the samples, it has its limitation. It is unable to analyse transitions involving multiple processes such as enthalpic relaxation which arises at the glass transition. Enthalpic relaxation is an endothermic process that varies in magnitude depending on the thermal properties of the

sample, which can, at times, make the glass transition appears as a melting transition. Such an issue is exacerbated when it is a mixture of multiple materials. Conventional DSC records the sum of all thermal events in the sample. Hence, multiple transitions occurring within the same temperature range, are difficult to separate. Temperature Modulated DSC (tmDSC) provides the same information as conventional DSC, as well as unique information of the materials, i.e. the reversing heatflow, that cannot be obtained from conventional DSC by overcoming most of its limitations. tmDSC separates the reversing events (i.e. heat capacity such as glass transition) from non-reversing events (kinetic events such as crystallisation and other first order phase transformation). The resultant heat flow at any point in a DSC or tmDSC experiment is described by the [Equation 3.16](#).

**Equation 3.16:** Heatflow Equation.

$$\frac{dQ}{dt} = C_p\beta + f(T, t)$$

where  $\frac{dQ}{dt}$  is total heat flow,  $C_p$  is heat capacity,  $\beta$  is heating rate and  $f(T, t)$  is heat flow from kinetic (absolute temperature and time dependent) processes.

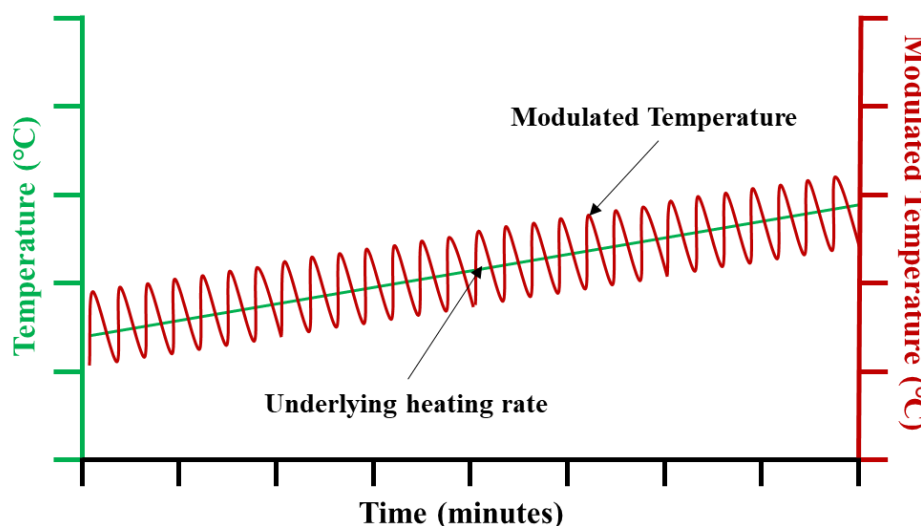


**Figure 3.21:** A typical tmDSC profile.

Just like conventional DSC, tmDSC measures the heatflow of a sample as a function of temperature and time, with the added factor of a sinusoidal modulation or oscillation. This oscillation is overlaid on the linear heating or cooling ramp to generate a profile where the average sample temperature continuously changes with time but not in a linear fashion, as demonstrated in the typical tmDSC heating profile in [Figure 3.21](#).

Imposing such a complex heating profile on the sample is the combination of two experiments, one is of that of a linear heating rate (in green) and the other is that of sinusoidal heating rate (in red), supplying information about heat capacity that responds to the rate of temperature change. The rates of the two simultaneous experiments are dependent on three variables, namely, underlying heating rate (1 to 10 °C min<sup>-1</sup>), period of modulation (40 to 100 seconds) and temperature amplitude of modulation (±0.5 to 2.5) °C, as tabulated in [Table 3.1](#). The heat capacity,  $C_p$  of the sample is continuously determined by dividing the modulated heat flow amplitude (measured in W g<sup>-1</sup> or mW) by the modulated heating rate amplitude (measured in °C min<sup>-1</sup>). In conventional DSC,  $C_p$  is generally calculated by two equations, with  $K_{C_p}$  as the calibration constant, one with the sample and a blank reference and the other by evaluating the difference in heatflow on identical samples heated at two different rates, as demonstrated in [Equation 3.17](#) and [Equation 3.18](#) respectively.

**Equation 3.17:** Heat capacity determination based on the sample and blank reference in conventional DSC at the same heating rate.

$$C_p = K_{C_p} \times \frac{\text{Heatflow}_{\text{sample}} - \text{Heatflow}_{\text{reference}}}{\text{Heating Rate}}$$

**Equation 3.18:** Heat Capacity determination based on identical samples heated at different rates, which produces different heatflows conventional DSC.

$$C_p = K_{C_p} \times \frac{\text{Heatflow}_{\text{Rate 2}} - \text{Heatflow}_{\text{Rate 1}}}{\text{Heating Rate 2} - \text{Heating Rate 1}}$$

However, the heating rate changes in the modulation cycle in tmDSC. The heat capacity is calculated in tmDSC in a manner where the measured amplitudes of the sample temperature and heat flow modulation are compared to a reference sine wave of the same frequency, in the [Equation 3.19](#).

**Equation 3.19:** Heat capacity determination in tmDSC technique.

$$C_p = K_{C_p} \times \frac{Q_{\text{amp}}}{T_{\text{amp}}} \times \frac{\text{Modulation Period}}{2\pi}$$

where  $C_p$  is the heat capacity  $K_{C_p}$  is the calibration constant,  $Q_{\text{amp}}$  is the heat flow amplitude,  $T_{\text{amp}}$  is the temperature amplitude.

**Equation 3.20:** Reversing Heatflow.

$$\text{Reversing Heatflow} = -C_p \times \beta$$

**Table 3.1:** A chart for the analyst to determine the maximum heat-only and period of modulation for respective underlying rate. For this project, an underlying heating rate of  $5\text{ }^{\circ}\text{C min}^{-1}$  was undertaken with a period of 60 seconds, which has a temperature modulation of  $0.796\text{ }^{\circ}\text{C}$ .

| <u>Heating rate (<math>^{\circ}\text{C min}^{-1}</math>)</u> | <u>Amplitude Period (seconds)</u> |           |           |           |           |           |           |            |
|--------------------------------------------------------------|-----------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
|                                                              |                                   | <u>40</u> | <u>50</u> | <u>60</u> | <u>70</u> | <u>80</u> | <u>90</u> | <u>100</u> |
|                                                              | <u>0.1</u>                        | 0.011     | 0.013     | 0.016     | 0.019     | 0.021     | 0.024     | 0.027      |
|                                                              | <u>0.2</u>                        | 0.021     | 0.027     | 0.032     | 0.037     | 0.042     | 0.048     | 0.053      |
|                                                              | <u>0.5</u>                        | 0.053     | 0.066     | 0.080     | 0.093     | 0.106     | 0.119     | 0.133      |
|                                                              | <u>1.0</u>                        | 0.106     | 0.133     | 0.159     | 0.186     | 0.212     | 0.239     | 0.265      |
|                                                              | <u>2.0</u>                        | 0.212     | 0.265     | 0.318     | 0.372     | 0.425     | 0.478     | 0.531      |
|                                                              | <u>5.0</u>                        | 0.531     | 0.663     | 0.796     | 0.929     | 1.062     | 1.194     | 1.327      |
|                                                              | <u>10.0</u>                       | 1.062     | 1.327     | 1.592     | 1.858     | 2.123     | 2.389     | 2.654      |

In tmDSC data, three heatflow thermograms are obtained, namely reversing heatflow, total heatflow and non-revering heatflow, as shown in [Figure 3.22](#). The heat capacity (reversing) component of total heat flow is calculated by converting the measured heat capacity into heat flow using [Equation 3.16](#) where  $\beta$  is the average heating rate used in the experiment and is obtained from the [Equation 3.20](#). The total heatflow is calculated as the average value of the measured modulated heatflow signal. The software is continuously calculating the average value rather than using only the two points of maximum and minimum per cycle. The non-reversing Heat Flow signal is the arithmetic difference between total heatflow and reversing heatflow.

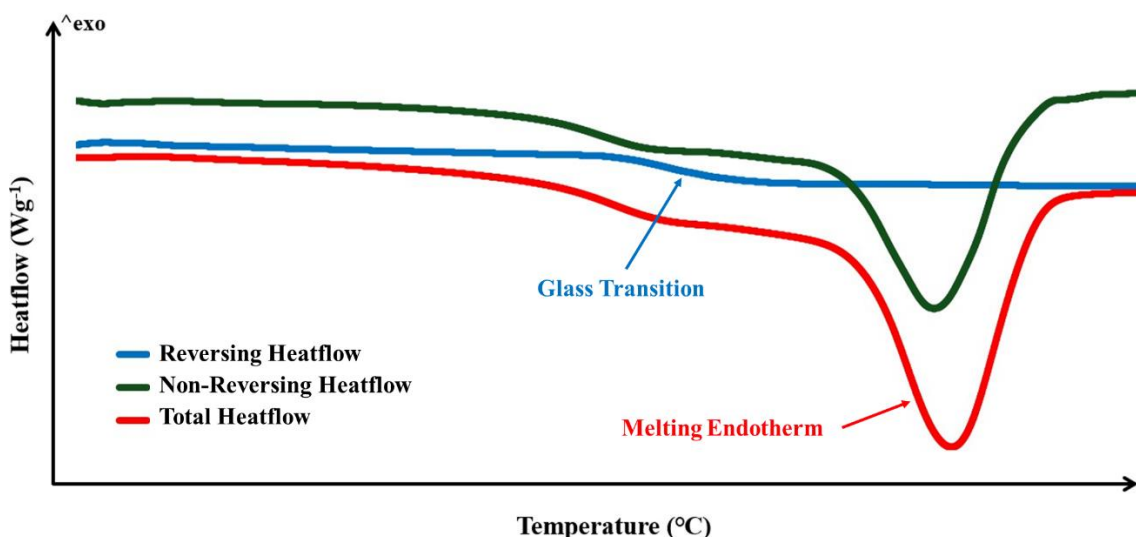


**Figure 3.22:** tmDSC plot of a semi-crystalline material, highlighting the glass transition event in the reversing heatflow and melting endotherm in the total heatflow.

The generation of a reversing heatflow thermogram in tmDSC makes this technique worthwhile for the analysis of amorphous materials. In a conventional DSC, the occurrence of multiple events within the same temperature range makes it difficult to interpret the heatflow signal. tmDSC generates thermograms of better resolution in terms of temperature as it utilises low heating rate that separates the overlapping transitions, making it easier to detect subtle transitions and analyse amorphous materials with much ease and accuracy. During the reheating of an amorphous material, an enthalpy overshoot occurs causing an artefact in the heatflow, due to physical ageing when stored below its glass transition temperature. Upon the imposition of sinusoidal modulation on the linear heating rate, tmDSC tackles this overshoot by separating the total heatflow into its reversing and non-reversing components, thereby resolving these overlaps. In doing so, the endothermic glass transition event reveals its presence in the reversing heatflow. This is an important thermodynamic event which relays the change in the material's specific heat capacity, which would otherwise be cloaked by the total heatflow signal. Furthermore, the clear appearance of the glass transition event in the reversing heatflow makes it easier to accurately quantifying the amorphous nature of the material.

For this project, both conventional DSC and tmDSC have been implemented to analyse the co-amorphous systems and their starting components. Two heating cycle were implemented in tmDSC to identify:

1. hydration and processing effects on the co-amorphous systems, and then
2. the composition of the anhydrous CAS that is formed.

A Mettler Toledo DSC1 STARe System (METTLER TOLEDO, Ohio, USA) was used to collect DSC data. Approximately 5 to 20 mg of sample was loaded into a 100  $\mu$ L aluminium crucible. The lid of the specimen holder was punctured to prevent pressure from building up within the crucible. An empty 100  $\mu$ L aluminium crucible was used as a reference. Samples were heated from 30 to 150  $^{\circ}$ C and then cooled back to 30  $^{\circ}$ C under a N<sub>2</sub> atmosphere at a flow rate of 50 mL min<sup>-1</sup> and at a temperature ramp rate of 10  $^{\circ}$ C min<sup>-1</sup>. Data collection was repeated in triplicate for each sample. Measurements to determine melting points ( $T_m$ ) and glass transition temperatures ( $T_g$ ) of the samples were performed using the Mettler Toledo STARe Evaluation Software 18.00 and were plotted with OriginPro 2023b (64-bit) SR1 10.0.5.157.

A Discovery DSC instrument (TA Instruments, Newcastle, USA) was used to carry out tmDSC, under N<sub>2</sub> atmosphere at a flow rate of 50 mL min<sup>-1</sup>, to determine the glass transition temperature ( $T_g$ ) of VAL and the co-amorphous systems under tmDSC. Approximately 2 to 10 mg of sample was loaded into an aluminium crucible with a volume of 40  $\mu$ L. The lid of the specimen holder was punctured to prevent pressure from building up within the crucible. An empty aluminium crucible was used as a reference. They were equilibrated at 0  $^{\circ}$ C for a couple of minutes and then heated from 0 to 150  $^{\circ}$ C, at a heating rate of 5  $^{\circ}$ C min<sup>-1</sup> and the temperature was modulated at an amplitude of 0.796  $^{\circ}$ C with respect to the heating rate, at a period of 60 seconds.

### 3.5.3. Microscopy

#### 3.5.3.1. Polarised Light Microscopy (PLM)

The optical microscope is used widely for pharmaceutical development with primary purpose of solid-state analysis. From illustrating and analysing particle sizes to observing crystallisation, the application of optical microscopy is significantly enhanced using polarised light. Polarised light microscopy provides an introduction to the internal structure of crystals in a manner that is aesthetically pleasing due to the vibrant colours and shapes of the crystals. It allows us to determine the optical crystallographic properties of the crystals (216).

A polarised light microscope has a polariser, an analyser, a compensator and Bertrand lens, alongside the standard components of a microscope such as eyepiece or ocular, objective lens, specimen stage, condenser, lens, diaphragm, adjustment knobs and a source of illumination (lamp) as shown in the [Figure 3.23](#). The polariser is located between the lamp and the specimen stage where the sample is placed. It restricts light

vibration to a single plane thereby creating polarised light. The analyser is positioned over the specimen which has its own polarising filter, which when rotated filters out the non-polarised light emerging out of the sample. When the vibration directions of the polariser and analyser are crossed, no light passes through, and the analyst's field of view is black. The specimen stage or plane is where the sample is placed for analysis. The compensator is placed between the analyser and the polariser to augment the resolution, contrast, and visibility of the sample by changing the optical path difference. A Bertrand lens is a specialised lens placed in between the objective lens and the ocular to observe the image of the objective's back focal plane into the plane of the ocular by projecting an interference pattern. The standard parts of the microscope include the ocular, which is the lens to observe the sample, the objective lens is a magnifying lens or a collection of lenses which can be rotated for varying magnification. The diaphragm controls the amount of light flowing through the set up. The lamp is the source of illumination, usually a tungsten bulb located at the base of the microscope and the condenser lens focuses the light onto the specimen.

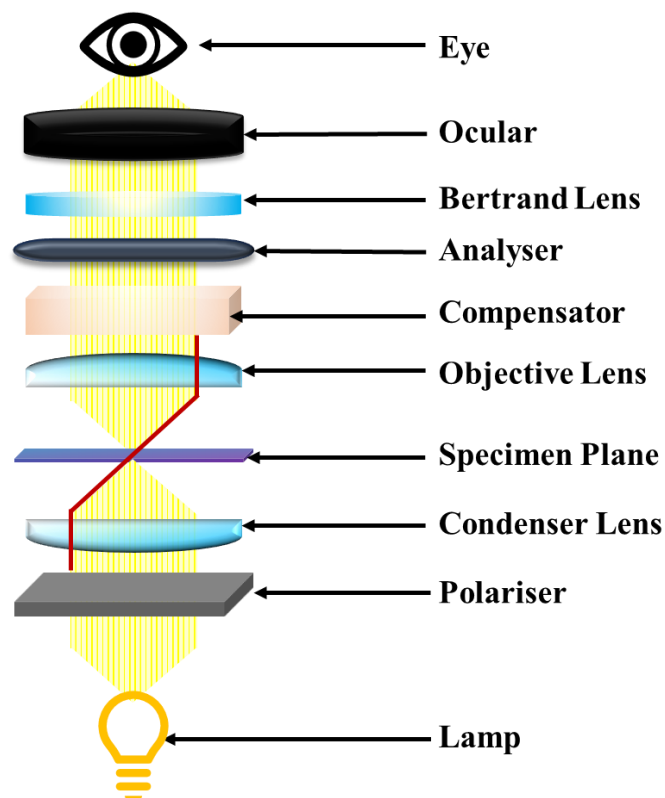


**Figure 3.23:** Schematic of a polarised light microscope.

The visible spectrum is a narrow part of the electromagnetic spectrum, which is visible to the human eye, extending from 380 nm to 750 nm in wavelength. The essential

characteristics of an electromagnetic radiation are frequency or wavelength, intensity, polarisation and phase and angular orbital momentum. PLM incorporates the functionality of the first four factors. The relationship between wavelength and frequency can be explained by the fundamental wave equation as shown in Equation 3.21, where  $\nu$  is the frequency,  $\lambda$  is the wavelength and  $c$  is the speed of the electromagnetic radiation.

**Equation 3.21:** Fundamental Wave Equation.

$$\nu = \frac{c}{\lambda}$$

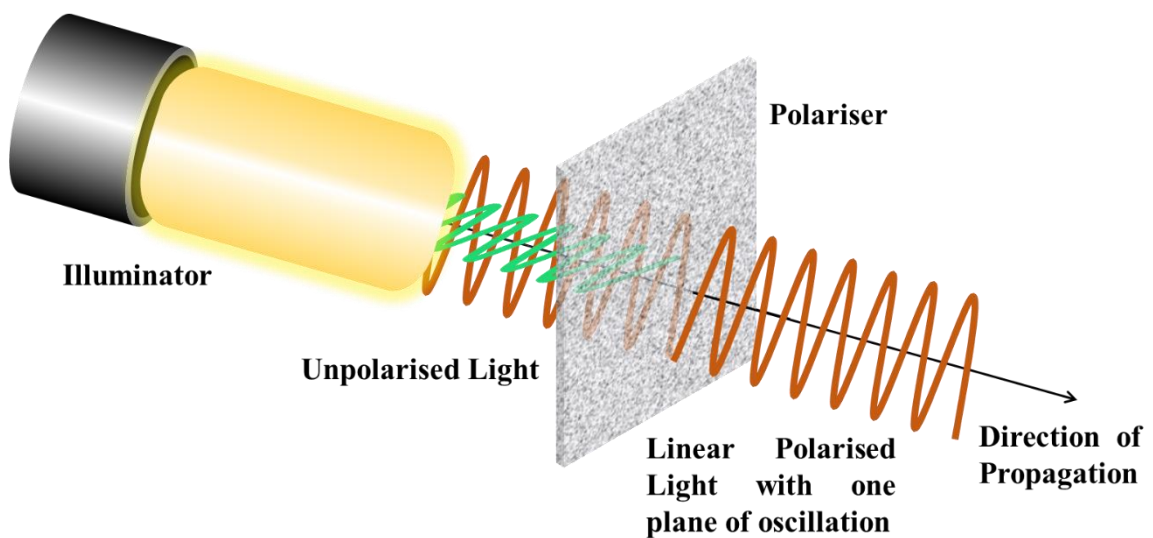


**Figure 3.24:** Polarisation of Light.

Polarisation is a property of transverse waves which imposes restrictions on the direction of wave oscillation. In such a wave, the direction of propagation of the wave is perpendicular to the direction of the oscillation. A polariser acts as a filter to allow light that oscillates only in one transverse orientation to pass through as shown in Figure 3.24. Birefringence or double refraction is the optical property of an anisotropic material where a ray of light splits into two components, each with different refractive index (ratio of the speed of light in vacuum to the speed of light in the material) and polarisation. Here anisotropy is measured as the difference between highest and lowest refractive indices of the material. When an unpolarised ray of light travels through the polariser, a linear polarised light which oscillates in a singular plane emerges. This polarised light transmits through a birefringent sample; the light ray further gets split into two parts which are perpendicular to each other. They interact differently with the material due to the different refractive indices, thereby travelling at different velocities through the material. Due to

the two different velocities of the two rays, their time of emerging out of the anisotropic material is different, thereby creating a phase shift or retardation or optical path difference (OPD) between them. This shift not only depends on the material's birefringence but also its thickness and the wavelength of the light. This light will pass through the analyser. In the absence of a birefringent material, this tool would block all light, and the field of view will be black. However, in the presence of birefringent material, the altered light components will get merged by the analyser, resulting in constructive and destructive interference thereby producing brightly coloured images revealing the structure of the material to the analyst who can then capture it on screen by a camera attached to the microscope. The specific colours generated can be quantitatively investigated (often using a Michel-Levy chart in [Figure 3.25](#)) to determine the degree of retardation and the material's properties.

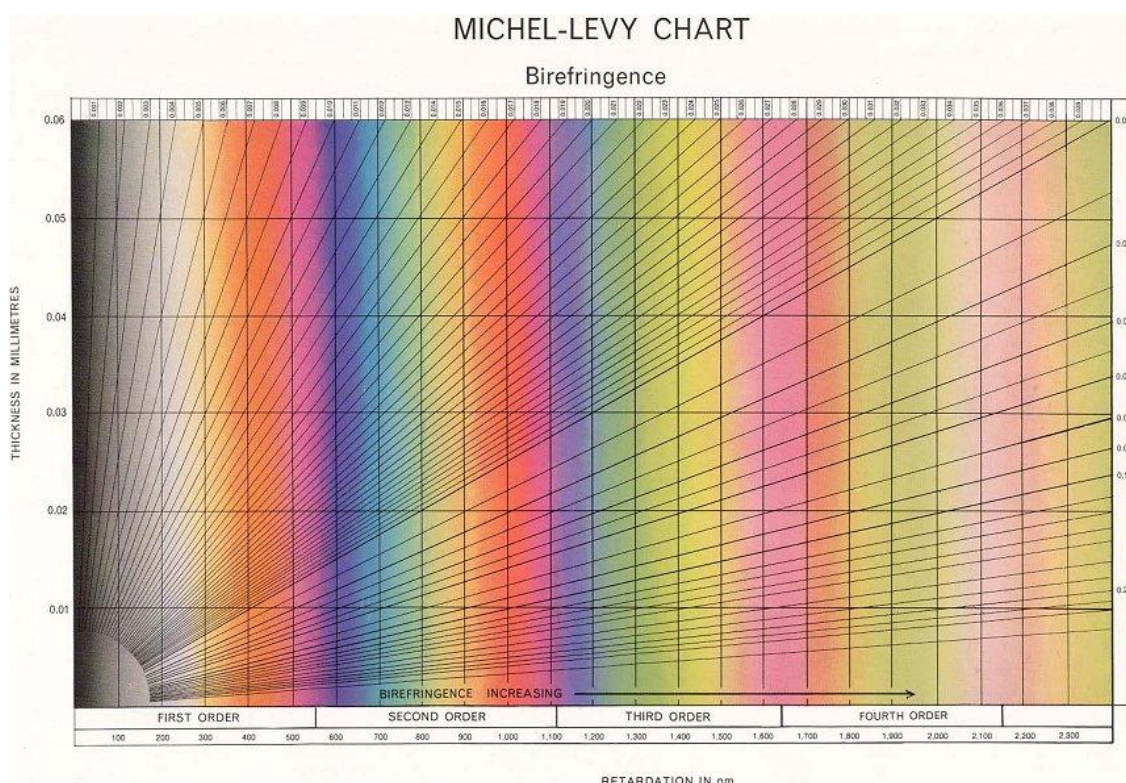


**Figure 3.25:** Michel-Levy Colour Chart. Image obtained from Dissemination of IT for the Promotion of Materials Science (DoITPoMS), a Creative Commons Attribution - NonCommercial-ShareAlike 4.0 International (217).

PLM is useful for determining if a sample is crystalline (birefringent or anisotropic) or amorphous (optically isotropic). It can assess the effectiveness of a preparation technique to prepare an amorphous system from a crystalline material or vice versa by assessing the net crystallinity of the sample by observing birefringence or lack

thereof. The resolution of PLM is limited to 300 nm but is able to detect nanocrystallinity without resolving them in an otherwise XRD amorphous system. Different polymorphs of a drug have varying solubilities, and PLM can distinguish these polymorphs on the basis of their unique morphology and optical properties (218-220). It can also identify impurities that may be present in a drug or drug delivery system, making it an important tool for quality control and assessment.

In this project, an Olympus BX51 Research System Microscope (Olympus Corporation, Tokyo, Japan) was used to collect images of the pure components when recrystallised in different solvents as well as in the solid-state co-amorphous systems. Cross polarised optical transmission microscopy was used to take images of co-amorphous systems in their dry, solid state. The images were taken with a AxioCam MRc 5 camera and analysed with the AxioVision and Fiji ImageJ 1.53c software.

### **3.5.3.2. Scanning Electron Microscopy (SEM)**

Scanning electron microscopy (SEM) utilises a focused beam of electrons to scan or study the surface morphology and composition of the sample being analysed and produce a high-resolution image of the sample. Scanning electron microscope generates images with much greater resolution than optical microscopes (221).

A scanning electron microscope is composed of an electron source, a vacuum system, the body of the microscope that comprises of anode, condenser lenses, objective lens and scanning coils and a sample chamber that contains a secondary electron detector, backscatter electron detector and stage, as depicted in [Figure 3.26](#). The microscopy occurs in a high vacuum environment, which is necessary for the electrons to travel down the length of the microscope to the sample to form an image without adverse scattering.

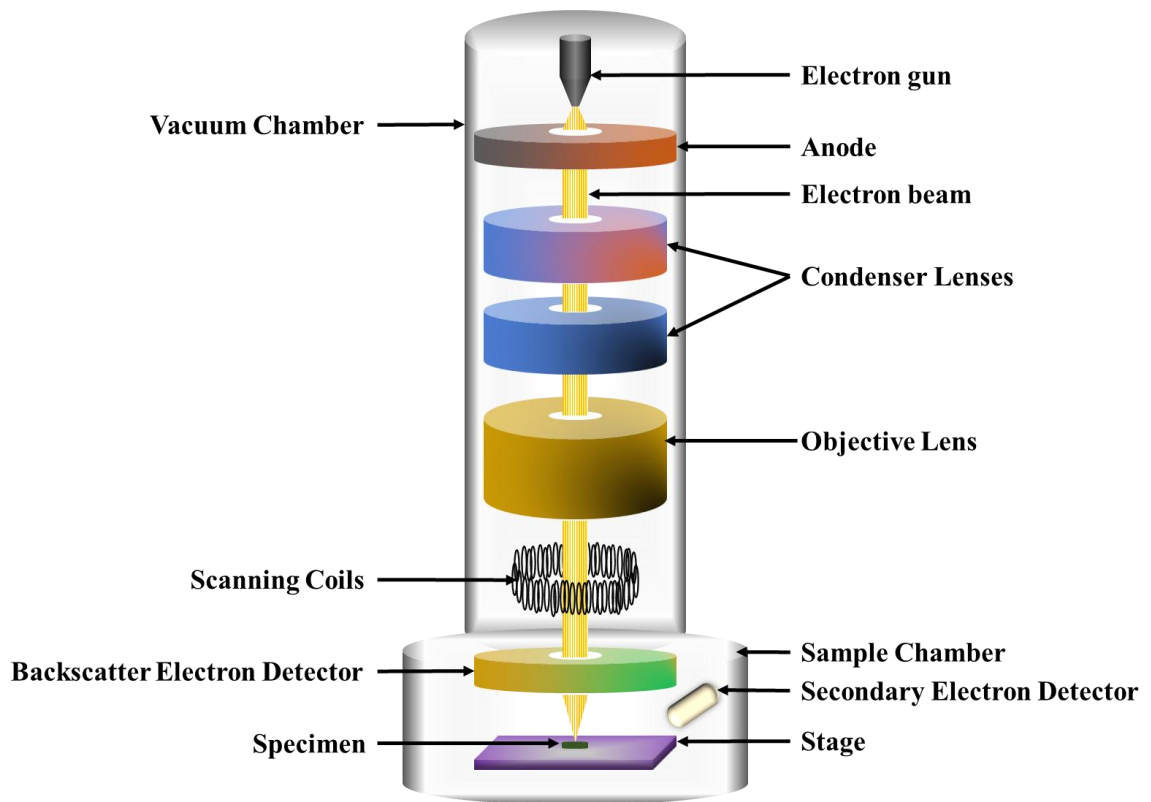


**Figure 3.26:** Schematic of a scanning electron microscope.

Electrons are produced at the top of the microscope by an electron gun such as field emission gun (FEG) or heated tungsten filament which are accelerated through the body of the microscope at a specified accelerating voltage (1 keV to 30 keV). Under the electron gun is the anode, which is a positively charged metal that attract the negatively charged electrons, accelerating them down the body of the microscope by forming an electron beam which passes through an aperture in its centre. The body of the microscope is essentially the column that contains the condenser lenses, the objective lens, and the scanning coils. The condenser or electromagnetic lenses control the size of the beam diameter by demagnifying the electron beam, ranging from less than 1 nm to 20 nm. The objective lens which focuses the beam to a fine spot on the sample surface. The scanning coils are electromagnetic and they deflect the electron beam by raster scanning technique across the sample, point by point. This leads to the formation of a detailed high-resolution image by building pixels one by one. The sample chamber has a secondary electron detector that detects electrons with low energies, usually between 0 to 50 eV, which are emitted from the surface or near to the surface (approximately less than 10 nm from the surface) of the sample thus giving knowledge about the topography of the sample whilst the backscattered electron detector detects the high energy incident electrons, usually higher than 50 eV and up to as that of the imaging beam, which are scattered back from

the sample. BSE can come from even deeper within the sample, and they supply information about the chemical composition of the sample. Finally, there is the stage where the sample is placed for analysis, which can be rotated, moved both horizontally and vertically and tilted for detailed imaging.

Just like optical microscopes use light as a wave, an electron microscope utilises the wavelike properties of an electron. However, the best resolution for a light microscope is 200 nm whilst that of a scanning electron microscope is 0.5 nm. Microscopic resolution can be obtained from the Rayleigh criterion, as shown in Equation 3.22, with  $\delta$  is the smallest resolvable distance,  $\lambda$  is wavelength of incident radiation,  $\mu$  is the refractive index of the viewing medium,  $\beta$  is the semi-angle of collection of light captured by the objective lens and 0.61 is the constant derived for circular apertures. The improved resolution of the SEM, which captures surface irregularities along with enhanced depth of field as well as the ability to conduct elemental analysis makes SEM a valuable technique for analysis in pharmaceutical research. Such detailed visual information gives the analyst information about how the physical form of a drug delivery system will affect its performance such as disintegration and dissolution. Furthermore, such a detailed observation can identify the presence of any defects or impurities, which can easily be picked up due to elemental analysis. It ultimately helps in the development of a formulation which is compliant with regulatory bodies.

**Equation 3.22:** Rayleigh Criterion Formula.

$$\delta = \frac{0.61\lambda}{\mu \sin\beta}$$

In this project, SEM was carried out to study the morphology of the co-amorphous systems. Macauley Hough carried out the SEM analysis after the samples were mounted onto an aluminium stub with a carbon sticker and air dusted to remove loose particles. They were then coated in chromium (to prevent electrical charge building up) in the Leica ACE600 EM coater (Danaher Corporation, Wetzlar, Germany). The Helios G4CX FIB-SEM (Thermo Fischer Scientific, Massachusetts, USA) was used to examine all the samples, operating at an accelerating voltage of 5 kV and probe current of 0.20 nA, with a focal length of 5 mm, equipped with Everhart-Thornley Detector (ETD) for Secondary Electron (SE) detection, under high vacuum. Images were taken at multiple magnifications for each sample. The images were analysed with Fiji ImageJ 1.53c software.

### 3.5.4. Infrared Spectroscopy (IR)

Infrared spectroscopy is the study of the bonding interactions within a compound or a group of compounds when irradiated with infrared radiation. The three main regions investigated by IR are the near-IR ( $14000\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$  or  $700\text{ nm}$  to  $250\text{ nm}$ ), mid-IR ( $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$  or  $2500\text{ nm}$  to  $25000\text{ nm}$ ), and the far-IR ( $400\text{ cm}^{-1}$  to  $10\text{ cm}^{-1}$  or  $25000\text{ nm}$  to  $10^6\text{ nm}$ ). The mid-IR region is of most interest as it contains the characteristic chemical bands for identification.

Raman spectroscopy is complementary to FT-IR, although it works on a separate principle to analyse molecular vibrations to IR spectroscopy. Raman spectroscopy will further be elaborated in section 3.5.4.2.

#### 3.5.4.1. Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy is a versatile, non-destructive analytical technique that identifies organic and inorganic compounds by recording their infrared light absorption. It operates by transmitting infrared light within the mid-infrared region of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ , through a sample and analysing how the varying wavelengths are absorbed depending on the fundamental vibrational and rotational states of the functional groups, thereby creating a unique "fingerprint" spectrum for each compound. FT-IR is convenient for identification, quality control, detection of impurities, and materials analysis, and is therefore a useful tool for pharmaceutical research.

An FT-IR spectroscope consists of an IR source, interferometer, sample stage, detector, and a data system as shown in Figure 3.27. The IR source produces the infrared radiation for the operation. The interferometer is the central component of the spectroscope consisting of a beamsplitter, a moveable mirror, and a fixed mirror. The beamsplitter is a partially reflecting mirror which "splits" the incoming radiation into two beams, one is reflected by a fixed mirror and the second by a moving mirror. They recombine at the beamsplitter after a path difference has been established by generating interference between the beams. Due to the changing travel distance of the second beam because of the moving mirror, the resulting IR light has a frequency distribution, leading to the formation of an interferogram, upon interaction with the sample. The detector receives the transmitted light from the sample and converts the transmitted IR radiation into a time-domain interferogram and implements a Fourier transform to convert this information into an infrared spectrum. The computer or the data system gathers the interferogram from the detector and produces a spectrum, which shows the wavelengths

absorbed by a particular molecule, usually around  $1800\text{ cm}^{-1}$  to  $600\text{ cm}^{-1}$ , all of which are unique to the molecule's composition and structure, thus becoming a “fingerprint” for the sample.

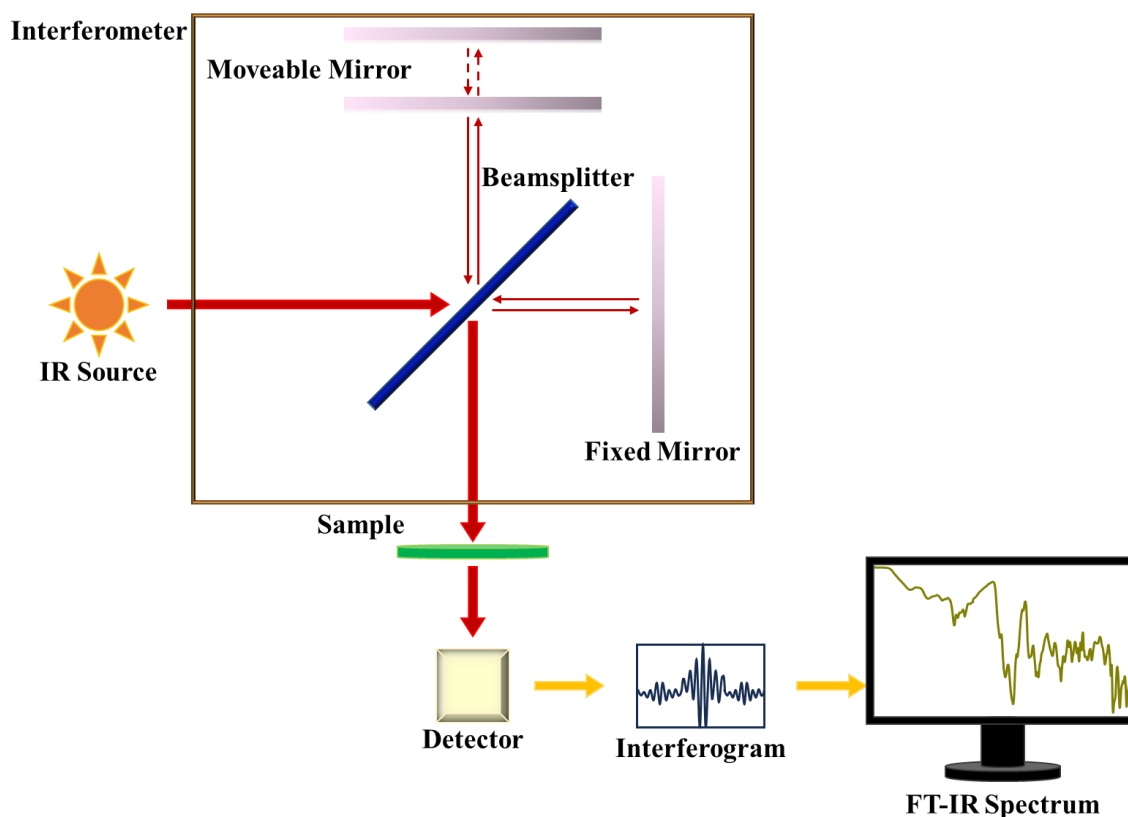


**Figure 3.27:** Schematic of an FT-IR spectroscope.

The IR spectrum can be depicted as a graph illustrating the intensity of transmitted infrared light versus the wavenumber of the transmitted infrared light ( $I$ ). Transmission is expressed as a percentage of the incident light  $I_0$ , as illustrated by the [Equation 3.23](#) and [Figure 3.28](#), where  $\epsilon$  is the molar absorption coefficient,  $c$  is the concentration of the substance and  $l$  is the path length the light travels through, which in this case is the thickness of the sample, based on the Beer-Lambert Law, where absorbance of light ( $A$ ) is directly proportional to the concentration of the sample and the path length of the light, as shown in [Equation 3.24](#) (222).

**Equation 3.23:** Equation for transmittance.

$$T(\%) = 100 \frac{I}{I_0}$$

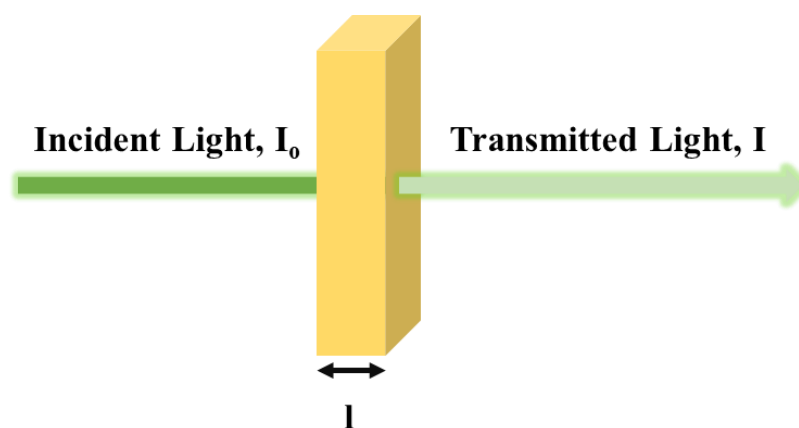


**Figure 3.28:** Transmission of light through a sample.

The absorbance has a logarithmic relationship to the transmittance; By applying a decadic logarithm transformation to  $T$ , the relationship between absorbance and transmittance is presented in [Equation 3.25](#).

The Fourier transform in FT-IR spectroscopy is a mathematical process that takes temporal data and generates a frequency spectrum. It deconvolutes the interferogram produced by the detector and sample for the wide range of infrared frequencies or wavelengths that were used at the same time to irradiate a compound to determine its chemical composition.

**Equation 3.24:** Beer-Lambert Law.

$$A = \epsilon cl$$

**Equation 3.25:** Relationship between absorbance ( $A$ ) and transmittance ( $T$ ).

$$A = \log_{10} \frac{I_0}{I} = -\log_{10} T$$

A Thermo Scientific™ Nicolet™ iS™ 10 FTIR spectrometer (Thermo Fischer Scientific, Massachusetts, USA), fitted with a single bounce attenuated total reflectance (ATR) sampling accessory, was used to obtain FTIR spectra. ATR utilises total internal reflection, where the IR beam passes through the ATR accessory/crystal and it interacts with the surface of the sample more than once, leading to the formation of evanescent wave (that does not propagate as a transmitted electromagnetic wave so that its energy is spatially focused on the interface and it penetrates the sample by up to 2  $\mu\text{m}$ ), as shown in [Figure 3.29](#). This enables molecular absorption and a spectrum of the sample to be produced (223).

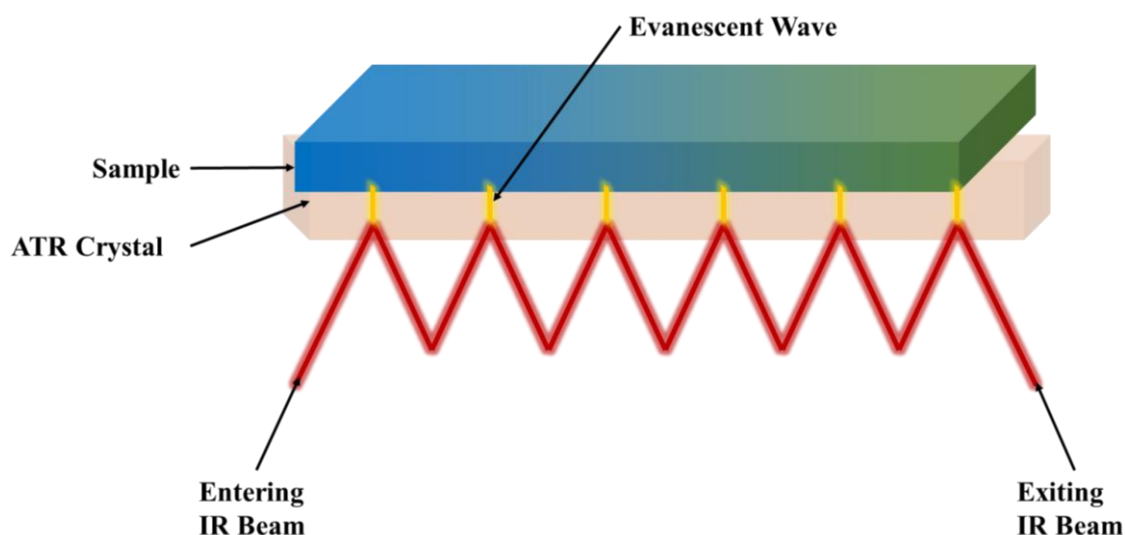


**Figure 3.29:** Pictographic representation of Attenuated total reflection (ATR).

Samples were loaded onto the ATR Diamond-ZnS and pressed down with a flat surface anvil to ensure proper contact between the sample and the crystal. Background spectra were collected for each sample before obtaining specimen spectra. Spectra were acquired in transmission mode (in percentage) within the range of 4000 to 600  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$  and averaged over 64 scans. Data collection was repeated in triplicate for each sample. The data were analysed with the Omnic software and were plotted with OriginPro 2023b (64-bit) SR1 10.0.5.157.

#### 3.5.4.2. Raman Spectroscopy

Raman spectroscopy is a non-invasive, rapid laser-based technique that utilises laser light to yield information about a sample's chemical structure and other properties. It works by irradiating a laser on a sample and analysing the scattered light, to generate a vibrational fingerprint or a Raman spectrum unique to the material's chemical composition and structure. In Raman spectroscopy, the laser interacts with the molecular vibration of the material and alters the energy of the laser photons and this change in energy provides information about the vibrational modes of the sample (224). This, like FT-IR, is useful in many industries including the pharmaceutical industry for quality control, identification of hydrates, detection of impurities for material analysis and especially for the monitoring spatial distribution of components in a formulation.

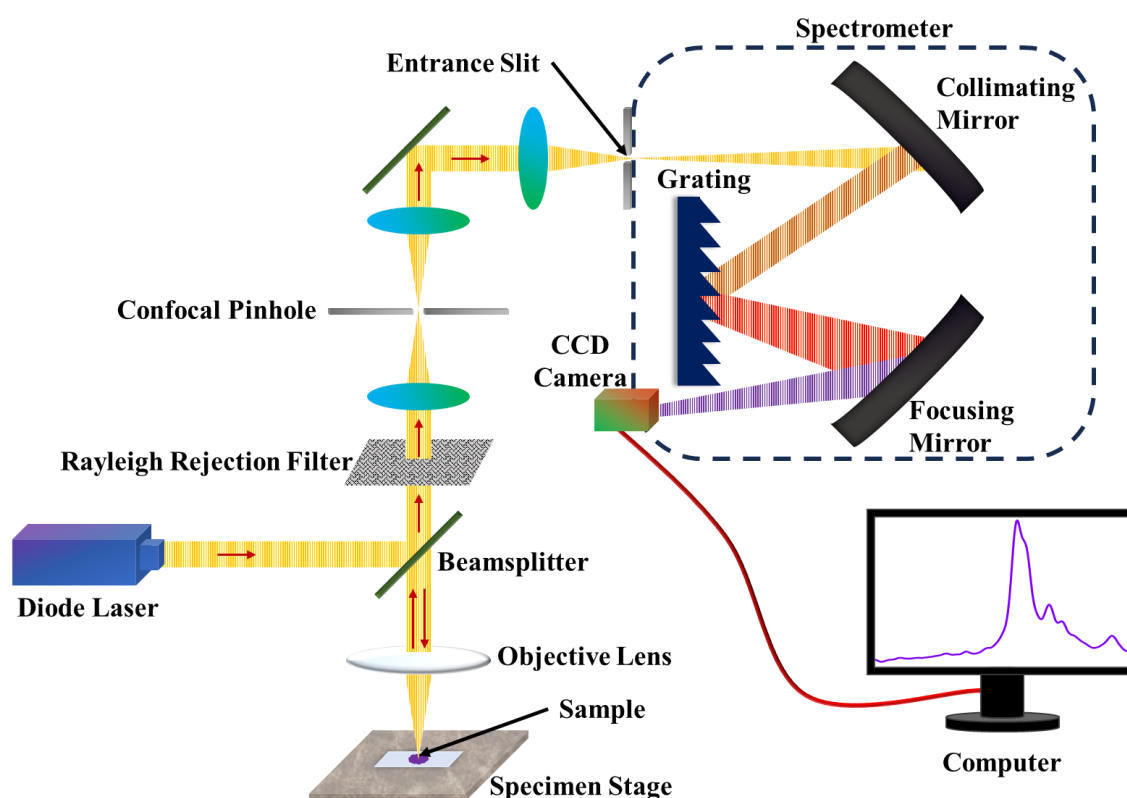


**Figure 3.30:** Schematic of a Raman spectroscope.

A Raman spectroscope is composed of a laser source, optical microscope, notch filter, a detector, spectrophotometer, and a data system, as demonstrated in [Figure 3.30](#). The laser source produces a beam of single wavelength to excite the sample. The wavelengths of the beam produced by the lasers are in the near infrared region ( $14000\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$  or  $700\text{ nm}$  to  $250\text{ nm}$ ), although the most used wavelengths are  $532\text{ nm}$  and  $785\text{ nm}$ . By increasing laser wavelength, the analyst can decrease the risk of fluorescence background interference. The objective lens focuses the beam onto the sample by a beam splitter and collects the scattering off the sample that goes through the beamsplitter to the Rayleigh rejection filter. This filter eliminates Rayleigh scattering (elastic scattering of photons, i.e., photons with high energy change) and only lets Raman scattering (inelastic scattering of photons) through. These filters are either made up of holographic films (notch filters) or optical fibres (edge filters). The confocal pinhole is a small aperture that acts as a spatial filter by blocking Raman scatter from anywhere other than the focal plane of the sample. Next in line on the optical path is the spectrometer which uses collimating and focusing mirrors and a diffraction grating to spatially split the constituent wavelengths of the Raman scatter and project them onto different pixels of a detector. The spectral resolution improves with an increase in density of the grooves of the diffraction grating however spectral range reduces with the increase in groove density.

Hence, multiple gratings are present in a spectrometer with different groove densities which can be accessed by a rotating turret depending on their experimental parameter requirement. Finally, the photons are detected by an array detector such as a CCD (charged-coupled device) camera that captures the light and converts it into a spectrum containing physiochemical properties of the sample under investigation, acting as its “fingerprint” and projects it onto the computer.

In this work, an inVia™ Renishaw confocal Raman microscope (Renishaw plc, Wotton-under-Edge, UK) equipped with a diode laser of 220 mW and 785 nm wavelength was used to obtain the Raman spectra of the co-amorphous systems. Samples were loaded directly on a slide, pressed down to a flat surface, and placed under 0.1% laser power and a 50 µm pinhole spectrograph aperture. The laser power at the sample surface was adjusted to around 0.22 mW as part of signal optimisation, to mitigate potential irradiation damage on the sample. Background spectra were collected every time for each sample before obtaining specimen spectra. Spectra were acquired in transmission mode (in percentage) within the range of 3200 to 400 cm<sup>-1</sup>, with a resolution of 4 cm<sup>-1</sup>. Data collection was repeated in triplicate for each sample. The data were acquired with the Renishaw’s WiRE™ 3.4 (Windows®-based Raman Environment) and analysed with Omnic 8.3.103 software and were plotted with OriginPro 2023b (64-bit) SR1 10.0.5.157.

### 3.5.5. High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a critical technique in analytical chemistry which separates, identifies, and quantifies compounds in a liquid mixture. These separations utilise the pressure-driven flow of a mobile phase through a column packed with a stationary phase. It has a wide range of applications in many industries, such as the food and beverage industry, pharmaceutical industry and even in environmental testing. HPLC precisely establishes the purity of APIs and final drug products by extricating components in the solution. Dissolution studies estimate how the ease with which an API is released into a solution, whether it is an aqueous solution or a simulated physiochemical solution i.e. for in-vivo or in-vitro studies. HPLC forces the solvent to travel through the column under high pressure whilst it identifies and quantifies the dissolving drug systems by a detector, making separation of the components swifter, thus giving the analyst some insights into the drug’s performance.

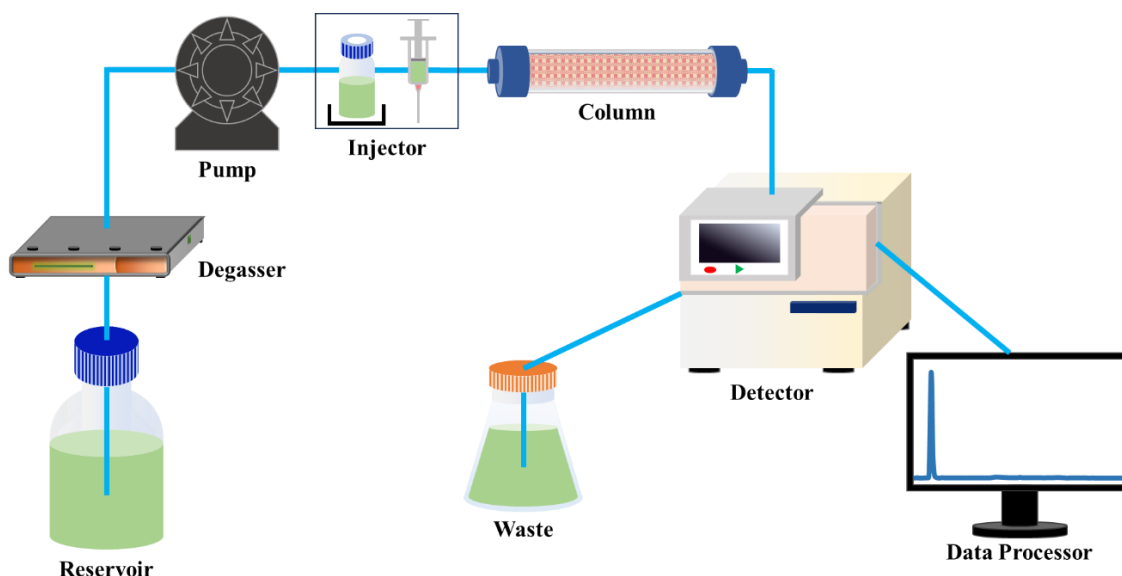


**Figure 3.31:** Schematic of a HPLC instrument.

A HPLC instrument is made up of a solvent reservoir, a high-pressure pump, a degasser, an injector, a column, a detector, a data system, and waste collector, as demonstrated in Figure 3.31. The solvent reservoir holds the mobile phase, i.e., liquid solvent or mixture of solvents that is pumped through the system at a controlled rate. The degasser removes dissolved gasses from the mobile phase, preventing the formation of bubbles that may interfere with the separation process. The high-pressure pump supplies the mobile phase at high, constant pressure and flow rate throughout the system. The injector incorporates a precise amount of the sample that is being analysed into the stream of the continuous mobile phase. The column, with length ranging from 5 to 250 mm, is the main part of this instrument where the separation occurs. It holds the stationary phase, the packing material that interacts with the sample. This packing material present in the column is typically made up of polymer resins or silica gels, and the size of these superficially porous particles ranges from 1.5 to 4  $\mu\text{m}$ . How the components in the sample separate solely depends on their interactions with the stationary and mobile phases. Some compounds are retained longer on the stationary phase, while others move through more quickly, thus the time taken by which each compound migrating from the beginning and end of the column (eluent or retention time) is different.

A detector measures the concentration of the compounds eluting through the column and signifies this to the data system. A detector can be a photodiode array (PDA) or an ultraviolet-visible (UV-Vis) spectrometer or a mass spectrometer. Finally, a computer attached to the chromatograph not only operates the instrument but also obtains

the data. The solution after the analysis is discarded into the closed-system waste collector equipped with activated carbon filters to trap volatile organic compounds (VOCs) as flammable, volatile solvents such as methanol, acetonitrile are used as solvents in the mobile phase in HPLC.

In this project, Dr. Jeanine Williams carried out the HPLC analysis by using an Agilent 1290 Infinity II HPLC system (Agilent, Santa Clara, CA, United States), with a diode array detector (DAD). Chromatographic separations were performed using an Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 50 mm, 1.9  $\mu\text{m}$ ) at a column temperature of 40 °C. The mobile phase used was 0.1 % trifluoroacetic acid (TFA) in water (95 %) and 0.1 % TFA in acetonitrile (5%) and the gradient running to 5 % water over 5 minutes at a flow rate of 0.5 mL/min. The DAD recorded the chromatogram at a wavelength of 254 nm. Calibration curve was generated and it was linear within a concentration range of 57.05–3423  $\mu\text{g mL}^{-1}$  for NIC and 19.15–766  $\mu\text{g mL}^{-1}$  for VAL. Information regarding HPLC parameters (chromatogram, retention time, limit of quantification are presented in [Appendix B](#)).

### 3.5.6. Equilibrium Solubility

Testing for equilibrium solubility is crucial in pharmaceutical research as it determines how an API performs in the body or in an aqueous solution to test the viability of different formulations. It not only identifies drugs with poor solubility early in product development, but also provides information required to formulate systems with better performance.

In this project, the shake flask method was used to determine the equilibrium solubility of the API and the co-amorphous formulations in Milli-Q water (pH 5.98) and phosphate buffer (pH 7.4), as shown in [Figure 3.32](#). The experiment was conducted at a controlled temperature of 25 °C by adding an excess amount of drug or binary system, above the absolute solubility limit, to 15 mL of solvent in 20 mL sample vials. The vials were placed in Sci-Quip Incu-Shake Maxi Benchtop Shaking Incubator (Rotherham, UK) and shaken for 24 hours at 250 rpm. It was left to equilibrate for 24 hours to allow the mixture to settle. After sedimentation, the supernatant fluid was collected, filtered through 0.22  $\mu\text{m}$  nylon filter and diluted into 2 mL HPLC vials for analysis via HPLC-PDA. All solubility experiments were performed in triplicate.

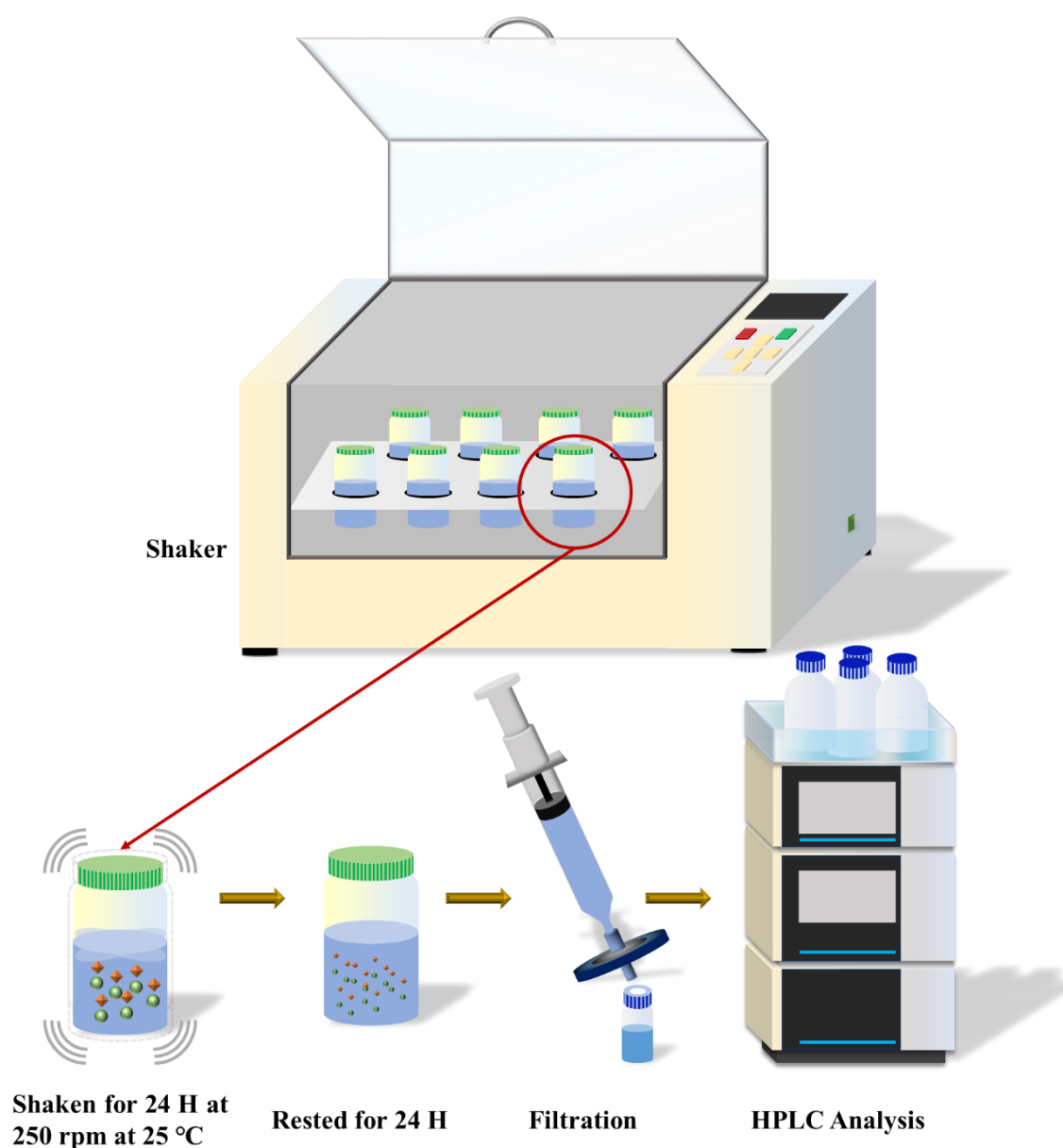


**Figure 3.32:** Schematic of determining equilibrium solubility by the shake flask method.

### 3.5.7. Conventional Dissolution Studies

Dissolution studies are of significant importance to the pharmaceutical industry to ensure drug systems are effective by estimating drug release from its dosage form. Such a study is used for quality control ensuring consistency in different manufacturing batches, identifying defects in the formulation as well as validating long-term stability of drug delivery systems. It can observe the influence of different preparation techniques on drug release to aid in formulation development to optimise drug release. Such a testing method is mandatory to obtain regulatory approval.

A conventional dissolution was carried out in this project by the sonication method. The ultrasonic waves accelerate the process of dissolving solids in a liquid by means of cavitation. Cavitation is a process where ultrasonic soundwaves create high- and low-pressure areas in the dissolving medium to create microscopic bubbles in the low-pressure areas which then collapse forcefully in the high-pressure areas and generates powerful shock waves. These shockwaves fragment the solid particles into much smaller particles, which significantly enhances their surface area exposed to the solvent. The increase in surface area increases the interaction between the solute particle with the solvent which enhances the dissolution rate significantly, as shown in [Figure 3.33](#).

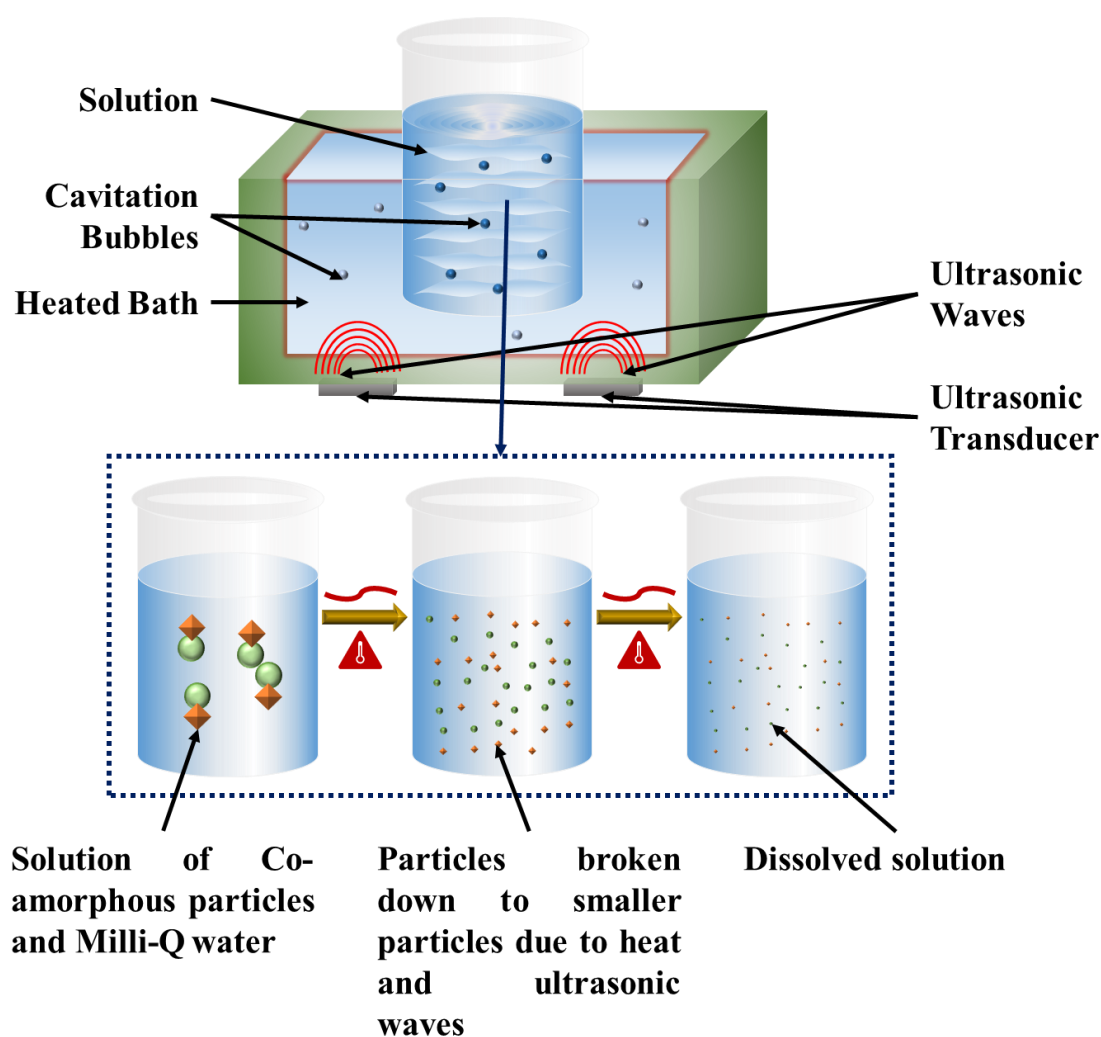


**Figure 3.33:** Schematic of ultrasonic dissolution.

In this work, intrinsic dissolution studies were conducted in a Shesto Ultrasonic Tank (Watford, UK). The experiment was conducted at a controlled temperature of 37 °C by adding an excess amount of solute to 100 mL of Milli-Q water in a 250 mL glass beaker. The beakers were then placed inside the sonication tank, and ultrasonic dissolution was performed in the delicate mode (runs at half power). 2 mL of solution was withdrawn at

pre-determined time intervals of 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 and 300 minutes. The aliquots were filtered through a 0.22  $\mu\text{m}$  nylon filter and diluted into 2 mL HPLC vials for analysis (7, 8, 38, 225). Equal volumes of Milli-Q water were immediately added into the dissolution media after removal of the aliquot. The equipment used for this experiment is shown in Figure 3.34. All dissolution experiments were performed in triplicate.

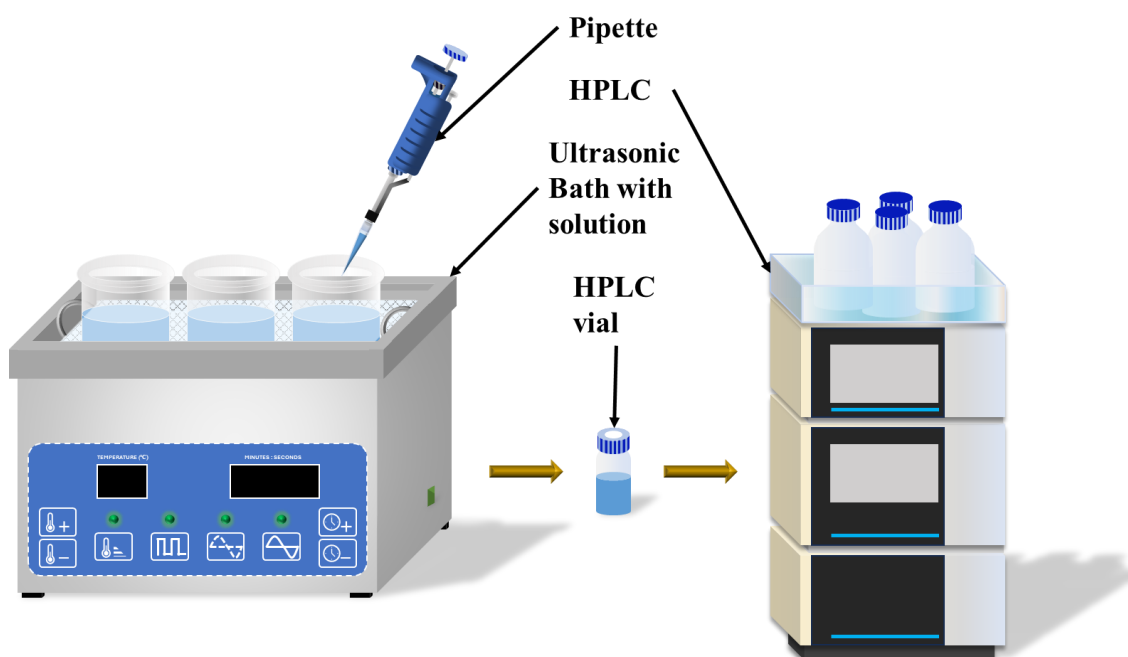


**Figure 3.34:** Dissolution was carried out in the ultrasonic bath, with 2 mL of aliquot of the solution withdrawn at regular intervals, filtered into HPLC vials and HPLC analysis was performed on them.

### 3.5.8. Pair Distribution Function

The pair distribution function (PDF) of a sample is derived from total X-ray scattering data which determines and provides quantitative information about the local atomic structure in the sample, specifically the arrangement of the data of nearest-neighbour atoms (226). In other words, PDF provides the probability of locating two atoms in a system, which are at a distance  $r$  apart and is dependent on the density of the atom pairs. PDF is useful for the study of crystalline, amorphous and liquid material (227). In conventional diffraction, only Bragg peaks are accounted for to determine structure whilst PDF analysis takes both the peaks as well as diffuse scattering between the peaks into account. The Bragg peaks disclose information about the long-range order of atoms present in a system, which is usually crystalline. PDF, on the other hand, also considers the diffused scattering, which contain information about the short-range order or the local atomic structure that is often less well-ordered. The diffused scattering that is

difficult to interpret in reciprocal space becomes simple to study upon the application of PDF analysis due to the transformation of the diffraction patterns to real space. This provides a host of information regarding bond lengths and structural disorder. PDF analysis is suitable for intrinsically disordered materials such as amorphous systems or nanomaterials such as APIs. The PDF can be a key identifier in distinguishing different amorphous states of the same material in the pharmaceutical industry, acting as a fingerprinting technique which can assess the APIs for quality control and identification.

The PDF,  $D(r)$ , is obtained from the experimentally determined total-scattering structure function,  $S(Q)$ , by a Fourier transformation demonstrated in [Equation 3.26](#).

**Equation 3.26:** PDF Equation. The PDF,  $D(r)$ , is determined by total-scattering structure function,  $S(Q)$ , by a Fourier transformation.

$$D(r) = \frac{2}{\pi} \int_{Q_{min}}^{Q_{max}} Q[S(Q) - 1] \sin(Qr) dQ;$$

$$= 4\pi r[\rho(r) - \rho_o];$$

Where  $Q$  is the magnitude of the scattering momentum,  $S(Q)$  is the total structure function obtained from Bragg and diffuse components of the X-ray scattering intensity,  $\rho_o$  is the atomic number density,  $\rho(r)$  is the atomic pair density.

The scattering data is collected by irradiating the sample with high energy X-rays (short wavelength) and the data are collected at very high scattering angles, with optimal background suppression. The data are then processed using Fourier transformation, as shown in [Equation 3.26](#) to calculate the PDF. The PDF pattern obtained is essentially a quantitative histogram that shows the probability of finding two atoms at a specific distance from each other. The position of the peaks depicts atomic distances, and the magnitude represents the likelihood of finding atoms separated by that distance. (228, 229). The pXRD pattern of an amorphous system has one or more characteristic “halos” which does not provide much of an insight into the structure of the amorphous system. Such systems are essentially disordered or have an arrangement of localised ordered domains, possessing short-range order (229, 230). Analysis of the PDF is useful for obtaining quantitative structural information from the amorphous sample in the absence of long-range order. Strong positive peaks provide information about the distances at which atoms are present and are likely related to structural ordering, specific to the sample. The troughs or negative peaks then represent distances at which no atom pair is defined. Peak widths provide insight about thermal and displacive disorder of the atoms.

The first few peaks denote nearest-neighbour or next-nearest-neighbour bonds. The broader intermolecular peaks at longer distances signify orientation and packing between molecules facilitated by hydrogen bonding or van der Waals forces.

PDF data for this project were collected at the I15-1 XPDF beamline at Diamond Light Source (Didcot, United Kingdom). Each sample was loaded into glass capillaries with a diameter of 1.0 mm. Data from the samples, empty instruments, and empty capillaries were collected at an energy of 76.69 keV ( $\lambda = 0.161669 \text{ \AA}$ ). The Q-range for the data was processed between 0.65 to 20  $\text{\AA}$ . Data normalisation and corrections for background, absorption, multiple scattering, and Compton scattering were conducted using GudrunX. Data, and were processed by Dr. Fanny Nascimento Costa via the Dawn Science software, analysed with Mercury 2024.3.1 and plotted with OriginPro 2023b (64-bit) SR1 10.0.5.157.

### 3.6. Computational Studies

Computational Studies are the application of computer simulations to model, simulate and analyse complex real-world systems to test and predict their functionalities and performance under different conditions. They can be carried out in a lab regulated computer or most commonly on high performing computer (HPC) clusters to analyse large datasets by using varying algorithms to develop models to predict their behaviour or solve complex problems efficiently. There are different kinds of computational studies; however, for this work computational chemistry has been undertaken to understand the functionalities of the co-amorphous form products of valsartan and nicotinamide.

Density functional theory (DFT) is a very popular, versatile computational quantum mechanical modelling method used to investigate the structure of molecules and their electronic and chemical properties and is widely used in computational chemistry and computational physics. In pharmaceutical research, DFT can be used for drug discovery and formulation, by investigating an API's electronic structure to understand its physiochemical behaviour, simulate reaction mechanisms, explore energy states and understand how it would interact with other drugs or proteins within the body as well as interaction with other components to study degradation pathways (10, 231, 232). Such a simulated study would be able to test the viability of formulation of APIs with varying excipient type and content in terms of stability, structural properties and performances for solubility, dissolution rates, and drug release kinetics. DFT analysis produces results that are swift and accurate, thereby cutting down expenses in terms of resources and time by

predicting outcomes and providing suitable excipients for APIs *in silico*. This can reduce the quantity of experiments required for novel drug discovery or formulation (233, 234). A semi-empirical method is a quantum chemistry method based on experimental data as well as theoretical calculation, thereby producing a system with improved accuracy in comparison to calculated data and experimental work. This is useful for developing an energy optimised model, which can then be utilised to produce simulated data for generating simulated spectroscopic or structural data, depending on the experimental requirements.

In this project, the computational infrared data of the co-amorphous system for comparison to experimental data was obtained through semi-empirical DFT calculations by the Gaussian 09 package using the HPC cluster at the University of Copenhagen (235). The molecular model of the individual components (VAL and NIC) and heterodimers of the components of differing supramolecular heterosynthons, was prepared and optimised by the Austin Model 1 or the AM1 semi-empirical method in GaussView 6.1.1 (236). The model IR spectra were generated through DFT calculation using the hybrid functional B3LYP combined with the 6-311++G(d,p) basis set, based on the work of Turek *et al* (10, 237). A scaling factor of 0.967 was included to compare the experimental and theoretical vibrational wavenumbers (238, 239). These molecular models were visualised using the GaussView 6.1.1 and OriginPro (2023b (64-bit) SR1 10.0.5.157) has been used to plot the spectroscopic data. This analysis assists in identifying the likely hydrogen bonding sites and changes in the IR of the components upon the formation of a single phase co-amorphous system.

## Chapter 4. Impact of molar ratio variation in the quality of co-amorphous systems of valsartan and nicotinamide

### 4.1. Chapter Overview

This chapter aims to identify the optimum molar ratio in terms of retained crystallinity, aqueous solubility and stability against recrystallisation in storage in high humidity for the production of a single phase co-amorphous system (CAS) formed between valsartan (VAL) and nicotinamide (NIC) based on the system prepared by Turek *et al* (10). The CASs of varying molar ratios were prepared by melt amorphisation and were analysed by structural, thermal, and spectroscopic techniques, all of which suggest that there is a lack of homogeneity across the compositional space, which may have an impact on the stability of the amorphous state of the system. Three model systems of the co-amorphous systems were designed via GaussView and DFT calculation indicated that two different heterodimers can exist between equimolar VAL and NIC, along with a third heterosynthon, which is a combination of those two heterodimers where one VAL is bonded to two molecules of NIC (235, 240). Equilibrium solubility of these binary systems in water (pH 5.98) was investigated and it was determined that equimolar CAS enhances VAL solubility the most but the absolute values of their solubility across the compositional space is not significantly different. The equimolar system had the highest physical stability against recrystallisation in comparison to the rest, when stored at an environment of 85% relative humidity, with all of them demonstrating an increase in crystallinity within a week except the equimolar system which maintained the least retained crystallinity, making 1:1 system the optimal ratio of the VAL-NIC co-amorphous system. This chapter highlights the influence of moisture on co-amorphous systems as well as providing identification of the optimal ratio of components in a co-amorphous system when held at high humidity at relatively brief storage times.

### 4.2. Introduction

An active pharmaceutical ingredient (API) defined as any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body (241). Such drug substances are broadly divided into

four categories based on their aqueous solubility and permeability Biopharmaceutical Classification System (BCS), developed from the work of Amidon *et al.* 1995, and it is implemented by the United States Food and Drug Administration as a regulatory framework for biowaiver approvals (1, 242). Approximately 90% of drugs in development and 40% of drugs on the market have poor aqueous solubility (2, 3). Inconsistent bioavailability and poor solubility of drugs are the main physiochemical properties that pose a challenge in the formulation of medications, especially in the cases of drugs belonging to the BCS Classes II (drugs with high permeability and low solubility) and IV (drugs with low permeability and low solubility) (4, 5). Solubility is a significant factor in the efficacy of a drug, thus poor solubility is a constraint in the formulation of effective drug systems (64). Furthermore, dissolution rate of a drug system is dependent on the solubility of the drug system, and has an impact on the bioavailability of a drug (65). Drugs with poor solubility lack the mobility to move into the blood stream, resulting in low bioavailability and low efficiency (66-68). The low solubility of APIs can be attributed to their crystalline structure, which possesses low energy and has low mobility (68).

To tackle this problem, crystalline drug forms can be transformed to amorphous counterparts which lack long-range order and periodicity (67, 68). Furthermore, amorphous forms of APIs have higher free energy, the molecules are randomly oriented and possess large surface area due to reduced particle size, all at which enhances wettability by increasing the exposure of functional groups (67). This can increase solubility and raise saturation concentration in solution which would increase dissolution rate (68, 72). Such an increase would enhance the bioavailability of the APIs with respect to their crystalline forms.

Amorphous forms are thermodynamically unstable and are prone to recrystallisation. Structural instability of amorphous APIs escalates when subjected to moisture because water behaves as a plasticiser to lower temperatures, thereby reducing the glass transition temperature ( $T_g$ ), changing molecular mobility and promoting recrystallisation (73). Hence, it is imperative to find a way of stabilising amorphous forms by introducing a secondary stability component to the API. A secondary component can be a polymer, an amino acid, a lipid or even another drug (8, 10, 73, 75, 76). A system with an API and a polymer is known as an amorphous solid dispersion (ASD) (75, 96-98). ASDs enhance solubility and stability of amorphous APIs, but they also reduce molecular mobility of the drug by the act of introducing intermolecular interactions

between the API and polymer. In addition, the hygroscopic nature of polymers can have a detrimental effect on the stability of ASDs as the systems may absorb more moisture than an amorphous API alone (102). Such sensitivity to moisture also has a negative influence over the miscibility between an API and a polymer matrix, (11, 96, 102, 103).

Co-amorphisation tackles the problems of recrystallisation and poor solubility of drug molecules. There are a couple of ways of formulating co-amorphous systems (CASs). An API can be combined with a low molecular weight co-former, such as an amino acid or another drug, to form a single phase homogenous amorphous system, (6-12, 68). These CASs have intermolecular interactions which increases miscibility, thus promoting higher levels of molecular mixing (12). This raises the activation energies against recrystallisation and phase separation, improving the overall physical stability of the drug system (12). Furthermore, the replacement of high molecular weight polymers with low molecular weight excipients has a favourable influence over the stability of the systems as it lowers the amount of excipient needed, thus reducing pill burden, as well as reducing the hygroscopicity problems that come with many polymers (11).

(S)-3-methyl-2-(N-(38-tetrazolyl-5-yl)biphenyl-4-yl-methyl)pentamido)butanoic acid or valsartan is an antihypertensive, BCS Class II drug, sold under the name *diovan*. It belongs to the angiotensin II receptor blocker (ARB) class of drugs. ARB is a group of pharmaceuticals that binds to and inhibit the hypertensive activity of Angiotensin II receptor type 1 (AT<sub>1</sub>). In other words, it relaxes and widens blood vessels, thereby lowering blood pressure. Persistent hypertension is a leading cause of cardiovascular diseases, especially heart attacks, strokes, heart failure and aneurysms (24). Developing better drug delivery systems with APIs that can tackle such a massive health problem is significant. Hence, it is important to develop a delivery system that has improved solubility and stability of VAL, as the efficacy of the drug is limited by its dissolution rate as the body cannot absorb it faster than the rate it dissolves (25).

Selecting a co-former to prepare a co-amorphous dispersion is not an easy task, as it has a strong influence on the stability and performance of the API in the delivery system (105, 136). In the preparation of CASs, the co-former must be generally recognised as safe (GRAS). In a drug-drug combination, both the APIs should be pharmacologically relevant to one another as well as having an interaction that enhances the stability and performance of the CAS (11, 12, 105, 136). In the case of a drug-excipient system, small molecules like amino acids or amides are a favourable option over polymers. Amides

lower bulk volume than polymer (68). They have higher solubility, whose presence in the CAS enhance the solubility of the API (11). Amino acids are classified as GRAS and are non-toxic, making them a suitable choice for co-amorphous formulations (68). Nicotinamide, also known as niacinamide, is a water-soluble form of vitamin B<sub>3</sub> (niacin), a nutraceutical with high bioavailability, is a suitable choice for CAS formation (29). It is an efficient, non-toxic hydrotrope that has excellent solubilising capabilities (14, 31, 108, 243). It has been reported to have several health benefits, such as supporting the cardiovascular system, acts as against inflammations, improves cognitive functioning, and reduces anxiety (153-155).

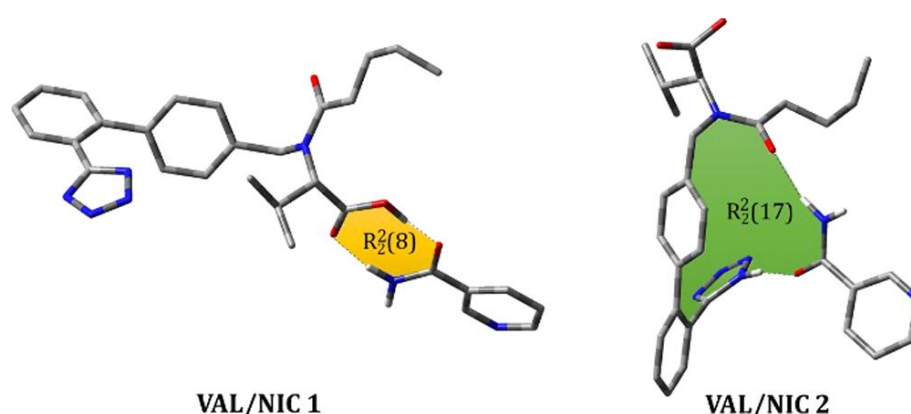


**Figure 4.1:** Supramolecular heterosynthons designed by Turek *et al* (1).

Turek *et al.* demonstrated that stable co-amorphous systems of VAL and NIC can be prepared. These systems are stabilised by hydrogen bonding between the two components in the form of two supramolecular heterosynthons as demonstrated by the [Figure 4.1](#). In the first heterosynthon VAL-NIC1, two hydrogen bonds can be observed between the two molecules, which are the N–H (NIC<sub>amide</sub>)–O (VAL<sub>acid</sub>) and O–H (VAL<sub>acid</sub>)–O (NIC<sub>amide</sub>) hydrogen bonds, thereby linking the VAL and NIC molecules into an acid–amide heterosynthon. In the second dimer VAL-NIC2, three functional groups are involved to form the two hydrogen bonds. The first one exists as the N–H (VAL<sub>tetrazolyl</sub>)–O (NIC<sub>amide</sub>) and the second one is present between O (VAL<sub>amide</sub>)–H–N (NIC<sub>amide</sub>). The work suggested that the occurrence of VAL-NIC1 heterodimer is more predominant than the other heterosynthon due to the similarity in the position of the calculated C=O stretching vibrations of VAL-NIC1, which is closer to experimental data in comparison to VAL-NIC2. Furthermore, computational studies by Turek *et al* suggests that the intermolecular hydrogen bond is the stronger in VAL-NIC1 (10). However, it does not rule out the possibility that both the heterodimers can co-exist in a co-amorphous system nor is it

possible to quantify the amount of each of the heterodimers existing within the said system.

Given the huge size of VAL molecule in comparison to the excipient NIC, it is a possibility that more than one molecule of NIC can bind with a singular VAL due to the multiple bonding sites in the drug molecule. The wider compositional range of VAL and NIC binary systems was not explored in the work of Turek *et al* (10). In this work, the wider compositional space has been explored by preparing multiple binary systems of the drug and the excipient. The properties of 16 co-amorphous systems of varying molar compositions of VAL and NIC (3.50: to 1:2.25) has been synthesised via the thermodynamic disarrangement method of melt amorphisation, as discussed in section 3.4.1. The resulting samples were stored in a desiccator (control group), and they were assessed for crystallinity and  $T_g$  by pXRD and tmDSC respectively on the day of the preparation. Determination of the deviation between the experimental  $T_g$  as determined by tmDSC and the theoretical  $T_g$  obtained by the Gordon-Taylor (GT) equation are presented to help in understanding of the composition of these binary mixtures (197, 198). The homogeneity, physical stability and the intermolecular interaction of the systems have been examined by powder X-ray diffraction, polarised light microscopy, FT-IR and Raman spectroscopy, and thermogravimetric analysis as discussed in Chapter 3 in section 3.5. Equilibrium solubilities of the systems with VAL and NIC molar ratios 2:1, 1.5:1, 1:1, 1:1.5 and 1:2 in water were determined. The systems with VAL and NIC molar ratios 2:1, 1.5:1, 1:1, 1:1.5 and 1:2 were aged at ambient temperature conditions of 85% RH. Powder X-ray powder diffraction and polarised light microscopy was implemented to observe physical stability against recrystallisation and increase in crystallinity in the co-amorphous systems during storage. The intermolecular interaction between the two components was further investigated by DFT calculations, as discussed in section 3.6 of Chapter 3, in the form of two binary heterosynthons as well as a third heterosynthon involving molecules which combines the aforementioned binary heterosynthons, to elucidate the potential bonding sites between the molecules (10).

### 4.3. Results and Discussion

Turek *et al.* demonstrated that stable co-amorphous systems of equimolar amounts of VAL and NIC can be prepared and these systems were characterised by spectroscopy, XRD and thermal analysis (10). Both components have functional groups, which can combine by intermolecular interactions to form stable heterosynthons (10). Chemometric

studies on the spectroscopic data were performed on these binary systems, which helped identify the different hydrogen bonds present between the API and the excipient, indicating that these bonds can enhance the stability of the two components against co-crystallisation (10). The  $\Delta pK_a$  values, as seen in [Table 2.3](#), of the investigated VAL-NIC system are less than zero, suggest the formation of hydrogen bonding is expected, and no salt formation shall occur within the systems.

The co-amorphous systems prepared by melt amorphisation have been investigated by PXRD to determine structure, tmDSC to determine the glass transition temperature, IR spectroscopy to study the molecular interaction between the two components, TGA to understand the thermal decomposition pathway and PLM to identify small-scale crystallinity and phase separation that may not be detected by PXRD. VAL, as received, is a white powder with a smooth texture. NIC, as received, appears to be very grainy and coarse. The melt amorphised (MA) CASs upon melting and cooling becomes completely transparent. However, upon running a spatula through the melted mass, it quickly breaks into a fine white powder, possibly suggesting crystallisation from a glassy state. This has been observed for all systems produced here.

#### **4.3.1. Powder X-Ray Diffraction (pXRD)**

The crystallinity of all samples after melt amorphisation was studied by pXRD. pXRD data of the as-received VAL suggests a partially crystalline material that can be accurately matched with the pXRD pattern reported by (244, 245). The crystalline fraction of the as-received VAL is evidenced by the presence of two sharp peaks at  $5.6^\circ$  and  $17.7^\circ$   $2\theta$  and the amorphous content by the broad peaks at  $15^\circ$  and  $25^\circ$   $2\theta$ . The pXRD patterns of VAL, NIC, and the co-amorphous systems across the compositional space of VAL:NIC 3.50:1 to 1:2.25, is presented in [Figure 4.2](#). pXRD data of NIC can be accurately matched to the simulated pXRD pattern generated from the Cambridge Structure Database (CSD – refcode NICOAM – deposition number 1219542) using the CrystalDiffract software (within the CrystalMaker suite) (246).

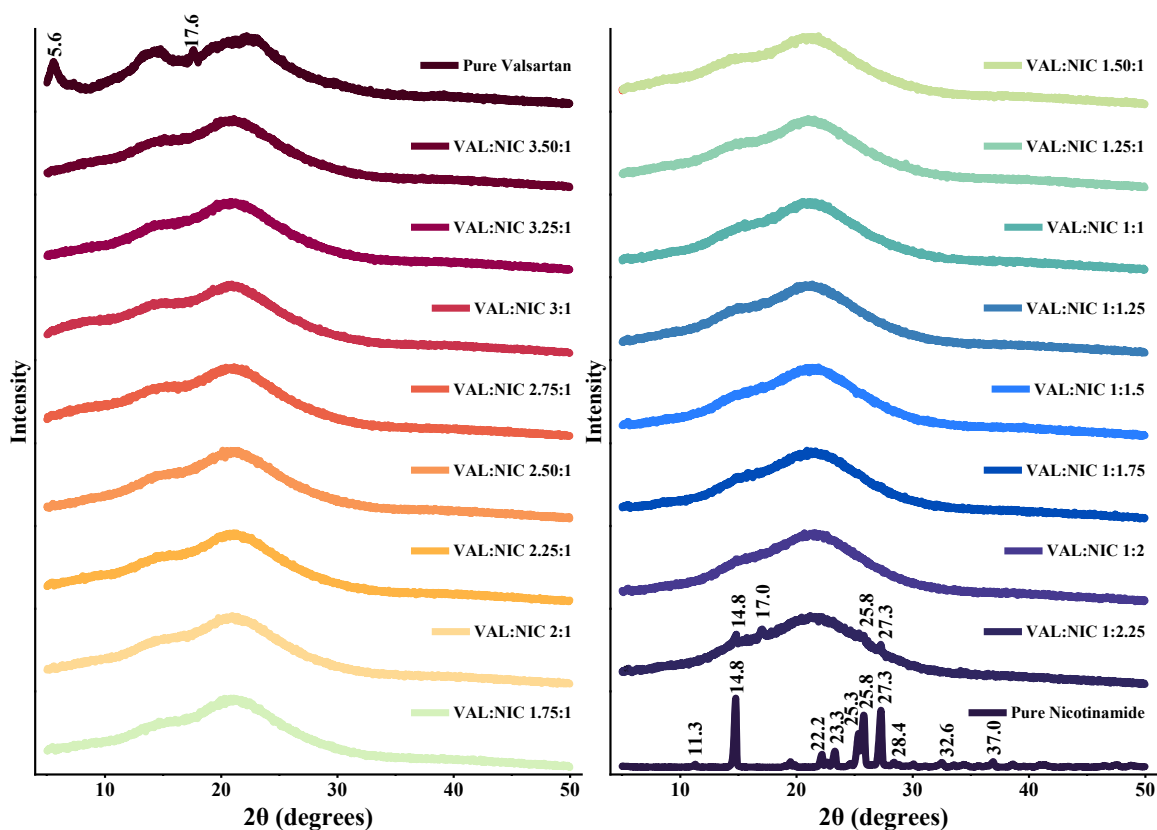


**Figure 4.2:** pXRD patterns of VAL and NIC as well as melt amorphised co-amorphous systems, ranging from ratios (VAL:NIC) 3.50:1 to 1:2.25.

The freshly prepared CASs are shown to be X-ray amorphous (up to a certain ratio) as evidenced by the diffuse peaks or an amorphous ‘hump’ in the pXRD patterns, with the absence of any crystalline peaks of either VAL or NIC. The CASs are X-ray amorphous between the molar ratios of 3.50:1 and 1:2 VAL:NIC content. Beyond the 1:2 ratio, the systems are no longer amorphous and contain four crystalline peaks in the pattern, three out of the four peaks are of monoclinic NIC (refcode NICOAM) and the peak at 17° is of VAL (244, 245). This suggests that the CAS possessing an excess of NIC (2.25 mmol) were highly unstable, resulting in recrystallisation of both the excipient and the drug; hence, no further CASs with higher NIC were prepared. pXRD patterns were obtained within hours of preparation.

### 4.3.2. Thermal Analysis

#### 4.3.2.1. Thermogravimetric Analysis (TGA)

TGA has been done to observe any changes in mass with increasing temperature and first derivatives generated to identify transition points which may not be observable in the as acquired thermograms.

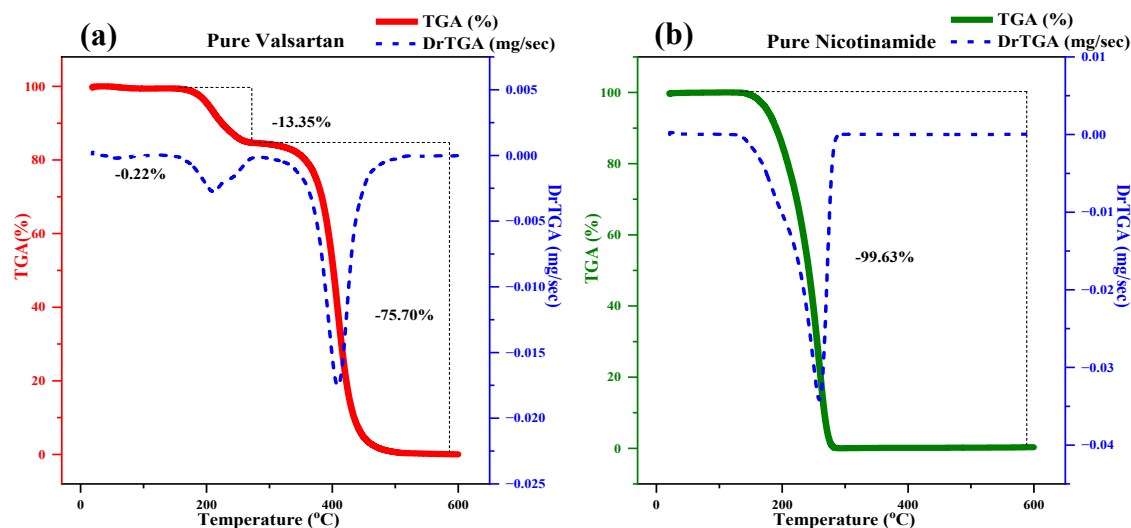


**Figure 4.3:** (a) and (b) TGA thermograms and first derivatives of pure valsartan and pure nicotinamide.

The TGA thermogram for as-received VAL in Figure 4.3 (a), demonstrates three mass loss regions. The first one occurs between 45 to 90 °C, with a meagre mass loss of 0.2%, followed by the mass loss of 13.4% between 155 to 270 °C, potentially due the decarboxylation of VAL, followed by the third and the largest mass loss of 75.7% between 270 to 600 °C. The first mass loss can be attributed to the loss of moisture absorbed by the VAL while the other two mass losses occur due to the thermal decomposition of the VAL (247-249). The TGA thermogram of NIC in Figure 4.3 (b), shows one mass loss event of 99.6%, that attributes to thermal degradation which occurs at 155 °C.

**Error! Reference source not found.** demonstrates the TGA thermograms of the MA CASs. The loss observed in the binary systems appears to follow the same pattern as that of as-bought VAL in Figure 4.3 (a). The TGA thermogram of MA CASs has three mass loss regions. The first one is a small mass loss region at 45 to 90 °C can be attributed to moisture loss. This mass loss due to the loss in moisture ranges from 0.08% to 0.8%, increasing with the increasing in VAL content, as depicted in the inset in **Error! Reference source not found..** The remaining two mass loss regions commencing a 155 °C and 270 °C are representative of that of VAL. However, as the amount of NIC increases, there occurs a shift in the mass loss amounts. The second mass loss region which starts at 155 °C gradually increases in loss with the increasing NIC, whilst

simultaneously the third mass loss region at 270 °C gradually decreases in loss, as expected with the increasing NIC content in the CASs.

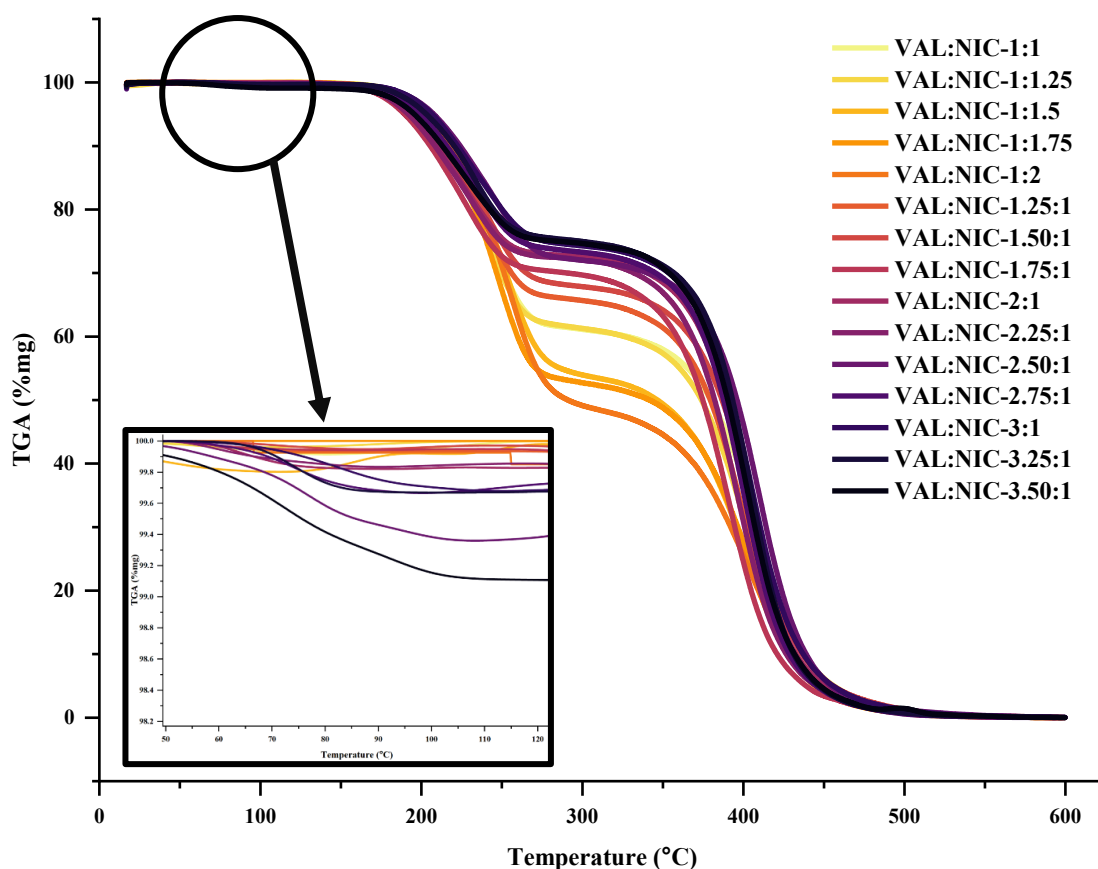


**Figure 4.4:** TGA thermograms of varying ratios of melt amorphised co-amorphous system. Inset: the small amount of mass loss due to moisture in the CASs.

Thus, TGA supports two findings. First, the small amount of weight loss due to moisture is present in all the CASs and it increases, ever so slightly, with increasing amount of VAL. Second, the mass loss reflects the composition of the systems, where systems with higher amounts of the drug follows the TGA pattern of the VAL whereas in systems with increasing amounts of NIC, the TGA follows the thermal decomposition pathway of the excipient.

#### 4.3.2.2. Differential Scanning Calorimetry (DSC)

The DSC thermograms of the pure components (VAL and NIC) and equimolar MA CAS are shown in Figure 4.5. The VAL thermogram showed 2 endothermic peaks: one a melting endothermic peak ( $T_m$ ) at 103.5 °C and the other a glass transition event at  $77.2 \pm 0.3$  °C, obtained by temperature modulated DSC, as explained in the next section (4.3.2.3.) The NIC thermogram displayed a singular endothermic peak at 129.7 °C, which

can be attributed to its melting point ( $T_m$ ). The equimolar MA CAS shows two endothermic events, one of which can be attributed to its glass forming ability and the second one, at 135.9 °C reflects the melting point of NIC. This melting event suggests the presence of unbonded, free NIC within the system, i.e. suggests a lack of homogeneity within the system.

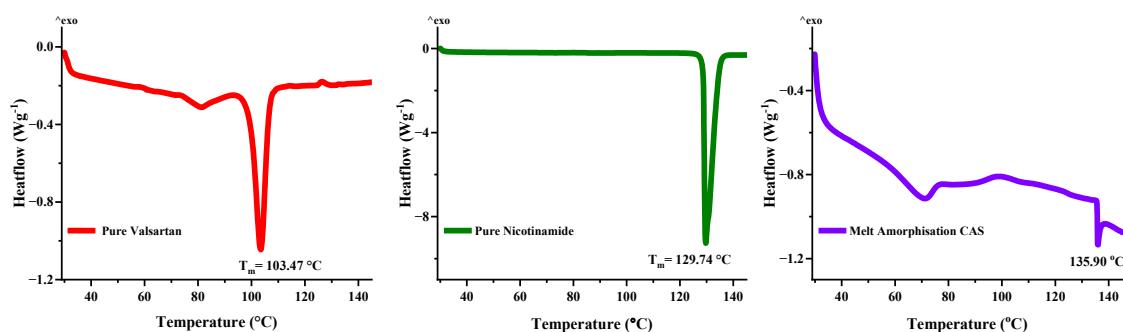


**Figure 4.5:** (left to right) DSC thermograms of valsartan, nicotinamide and equimolar melt-amorphised co-amorphous system.

#### 4.3.2.3. Temperature Modulated Differential Scanning Calorimetry (tmDSC)

tmDSC divides the total heat-flow rate, the one usually observed in normal DSC, into two heat-flows, namely reversing and non-reversing heat-flows to better separate kinetic events like crystallisation or enthalpy relaxation effects from reversible, heat capacity changes.  $T_g$  is determined by analysing the reversing heat-flow (250).

The Gordon Taylor (GT) equation has been used extensively to analyse and determine the glass transition temperature ( $T_g$ ) of binary co-amorphous systems (36, 149). It is crucial to understand the relationship between the  $T_g$  of a binary system and the molar ratio of its components, as it is may sometimes be difficult to produce a homogeneous amorphous system. To evaluate the theoretical  $T_g$  using the Gordon-Taylor equation,  $T_g$ s of the individual components within a co-amorphous system, must be known.  $T_g$  of VAL has been experimentally determined in this study to be  $77.2 \pm 1.3$  °C ( $350.2 \pm 1.3$  K) by tmDSC as well as standard DSC, as shown in Figure 4.5. For NIC, its amorphous form was highly unstable as it recrystallised rapidly even when undergoing quench cooling (at room temperature) after heating it at a temperature above its melting point (data not shown). However, Zhou *et al* had determined its  $T_g$  to be  $-7.3 \pm 0.2$  °C ( $265.7 \pm 1.3$  K), when preparing a fully amorphous NIC by quenching the drug melt in liquid nitrogen (149, 251).

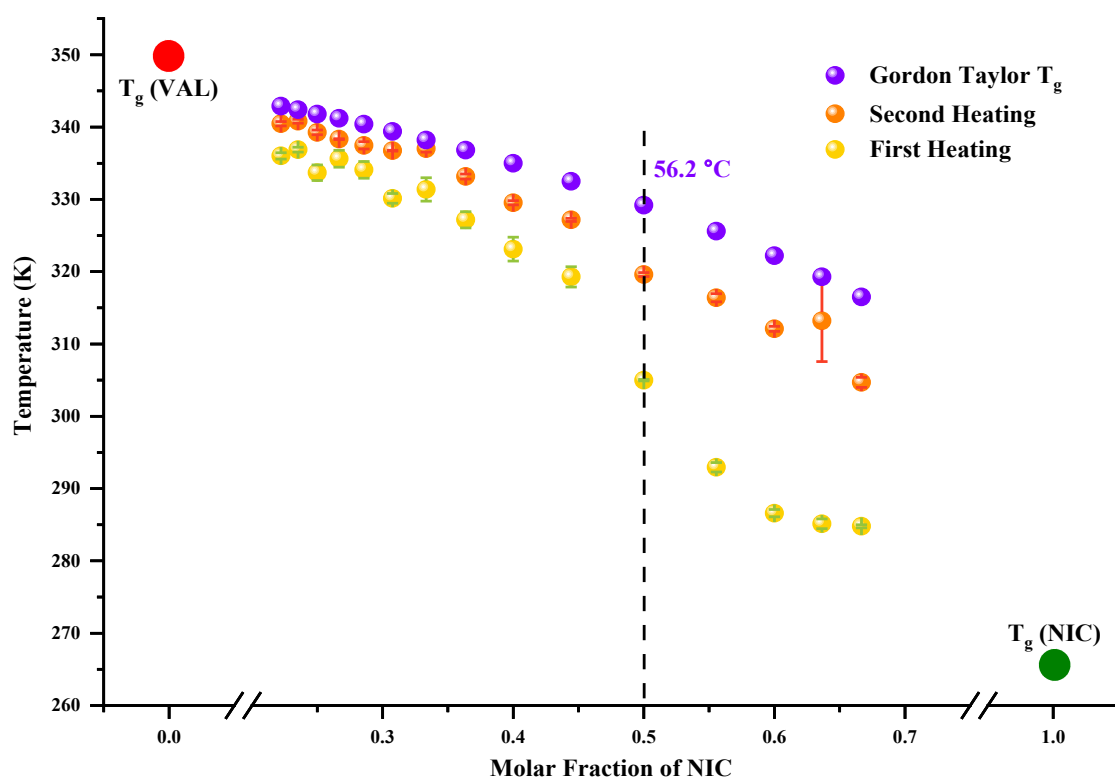


**Figure 4.6:** Comparison of Gordon Taylor and Experimental Glass Transition Temperature for 1st and 2nd heating cycles. The  $T_g$  of VAL and NIC are depicted in red and green, respectively. Each experimental data point is an average of three repeats along with a standard deviation depicted by the error bars.

After the confirmation of successful amorphous phase formation of the varying ratios of the VAL:NIC CASs by pXRD, the  $T_g$ s of the systems were investigated by means of tmDSC. [Figure 4.6](#) and [Error! Reference source not found.](#) report both experimental and theoretical  $T_g$  values. The  $T_g$ s (both experimental and calculated) have been plotted as an increasing weight percentage of NIC, from 20% to 70%, within the CASs of different molar ratios. A second cycle was employed to obtain the anhydrous  $T_g$  values of these CASs of varying molar ratios. The heating rate was  $5\text{ }^{\circ}\text{C min}^{-1}$  and the temperature was modulated at an amplitude of  $0.796\text{ }^{\circ}\text{C}$  with respect to the heating rate, at a period of 60 seconds for both the cycles. As amorphous NIC has high crystallisation tendency, it is practically impossible to determine the experimental  $T_g$ s of VAL-NIC co-amorphous systems with molar ratios of NIC higher than 0.67 (VAL:NIC ratio of 1:2.25).

**Table 4.1:** Tabulated  $T_g$  (calculated and experimental – 1st and 2nd Heating) values of the co-amorphous systems between the ratios (VAL:NIC) 1:2 to 3.50:1, with respect to the excipient and drug weight fractions. The colours in the table reflect the data points in the **Figure 4.6**.

| <b><u>Molar Ratios (VAL:NIC)</u></b> | <b><u>Experimental (<math>T_g</math>) (K) for 1<sup>st</sup> Heating</u></b> | <b><u>Experimental (<math>T_g</math>) (K) for 2<sup>nd</sup> Heating</u></b> | <b><u>Calculated <math>T_g</math> (K) by GT</u></b> | <b><u>Weight % of NIC</u></b> | <b><u>Weight % of VAL</u></b> |
|--------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------------------------|-------------------------------|-------------------------------|
| 1:2                                  | 284.8±0.2                                                                    | 304.7±0.7                                                                    | 316.6                                               | 0.67                          | 0.33                          |
| 1:1.75                               | 285.1±0.7                                                                    | 313.2±5.6                                                                    | 319.3                                               | 0.64                          | 0.36                          |
| 1:1.50                               | 286.6±0.5                                                                    | 312.1±0.4                                                                    | 322.2                                               | 0.6                           | 0.4                           |
| 1:1.25                               | 292.9±0.7                                                                    | 316.4±0.6                                                                    | 325.5                                               | 0.56                          | 0.44                          |
| 1:1                                  | 305.0±0.1                                                                    | 319.6±0.3                                                                    | 329.2                                               | 0.5                           | 0.5                           |
| 1.25:1                               | 319.3±1.4                                                                    | 327.2±0.2                                                                    | 332.5                                               | 0.44                          | 0.56                          |
| 1.50:1                               | 323.1±1.7                                                                    | 329.5±0.3                                                                    | 335.0                                               | 0.36                          | 0.64                          |
| 1.75:1                               | 327.2±1.1                                                                    | 333.2±0.4                                                                    | 336.8                                               | 0.33                          | 0.67                          |
| 2:1                                  | 331.4±1.6                                                                    | 337.0±0.5                                                                    | 338.2                                               | 0.31                          | 0.69                          |
| 2.25:1                               | 330.1±0.7                                                                    | 336.7±0.1                                                                    | 339.4                                               | 0.29                          | 0.71                          |
| 2.50:1                               | 334.1±1.2                                                                    | 337.5±0.5                                                                    | 340.3                                               | 0.27                          | 0.73                          |
| 2.75:1                               | 335.6±1.2                                                                    | 338.3±0.1                                                                    | 341.2                                               | 0.25                          | 0.75                          |
| 3:1                                  | 333.7±1.1                                                                    | 339.3±0.3                                                                    | 341.8                                               | 0.25                          | 0.75                          |
| 3.25:1                               | 336.9±0.3                                                                    | 340.8±0.3                                                                    | 342.4                                               | 0.24                          | 0.76                          |
| 3.50:1                               | 336.0±0.4                                                                    | 340.5±0.3                                                                    | 342.9                                               | 0.22                          | 0.78                          |

The first heating cycle tracked events such as CAS hydration with lower-than-expected  $T_g$ s, isolated components with VAL or NIC  $T_g$ s and component crystallisation with VAL or NIC  $T_m$ s, and they are tabulated in [Appendix A](#).  $T_g$  values reflecting amorphous VAL were observed in systems with the VAL:NIC ratios 1:2, 1:1, 1.50:1 and 1.75:1. Additionally,  $T_m$  reflecting crystalline VAL was present in systems of 1:1.50, 1:1.25, 1:1 and 3.25:1 and that of NIC in the systems of 1.50:1, 1.75:1 and 2.25:1. The

occurrence of multiple phases indicate that the as-prepared systems are not completely homogeneous. There are traces of unbonded components across the compositional space, some of which have also recrystallised. Systems with higher VAL content appear to have more unbonded crystalline NIC present in them and vice versa. This is possibly due to the fact that the binary systems on either end of the compositional space are overloaded with one phase that self-binds leaving behind excess molecules that cannot bond effectively throughout the system. The equimolar system indicates the presence of some unbonded VAL in both amorphous and crystalline form, indicated by an additional  $T_g$  at  $66.7 \pm 1.3$  °C ( $339.7 \pm 1.3$  K) and a melting endotherm at  $108.2 \pm 0.6$  °C ( $381 \pm 0.6$  K). The occurrence of VAL crystallinity is evident by PLM analysis in the equimolar CAS, despite having the least retained crystallinity, that is discussed in section 4.3.3. The first heating also shows a downward trend in the dominant  $T_g$  of each CAS with increasing NIC content that increasingly deviates further below the  $T_g$  predicted by GT equation as shown in Figure 4.6 and in **Error! Reference source not found..**  $T_g$  is heavily influenced by the water content in these systems because amorphous products can be hygroscopic, and the presence of moisture may lower the glass transition of these co-amorphous systems due to the plasticising effect of water on NIC, if not both NIC and VAL (142, 149, 252-254). The presence of moisture is confirmed by TGA (**Error! Reference source not found.**) which appears to be associated with the VAL content of each CAS. Regardless, the presence of water in the CASs is further confirmed by the raising of  $T_g$ s on the second heating cycle, close to the values expected from the anhydrous GT equation as also shown in Figure 4.6 and in **Error! Reference source not found..** The highly polar nature of NIC makes it very susceptible to higher plasticisation by water, compared to VAL, as the amide group in NIC bonds easily with water. The water molecules can insert themselves between the molecules, forming a network of intermolecular hydrogen bonds and increase the molecular mobility of NIC molecules, leading to the onset of phase separation and even recrystallisation in a CAS even without the other component plasticising significantly (149).

Another reason for higher  $T_g$  values for the second heating could also be due to the further mixing of the components, resulting from the first heating cycle, thereby enhancing the homogeneity within the system. However, after the second heating, the experimental  $T_g$  values of the systems followed the same downward trend of the theoretical anhydrous  $T_g$ s, suggesting that dehydration is the most significant first heating cycle effect.

Thus, tmDSC provides two sets of  $T_g$  values from two heating cycles, both of which are lower than the calculated GT values.  $T_g$  values from the first heating cycle diverges more from the calculated values in comparison to the second heating cycle. This can be attributed to the moisture loss in the first heating as well as further mixing of the components during that cycle, thus increasing the  $T_g$  values of the second heating cycle. Moreover, as the NIC content increase, the  $T_g$  values shift further away from the GT values in both the heating cycles. This divergence is due to the presence of amorphous NIC that bonds with water easily in comparison to VAL, especially in the as-prepared system, driving the  $T_g$  of the as-prepared samples closer to the  $T_g$  value of hydrated amorphous NIC, which can potentially lead to phase separation and recrystallisation. Additionally, the first heating cycles observed additional endothermic events of melting and glass transition randomly across the compositional space which indicates some minor phase separation and recrystallisation, possibly due to inhomogeneous mixing or trapped moisture or both.

#### 4.3.3. Polarised Light Microscopy (PLM)

Polarised light microscopy (PLM) provides us insight to any small fractions of crystallinity of the systems, namely that of the pure components and within the co-amorphous systems themselves. Crystallinity was observed in the form of birefringence within the solid amorphous particles.

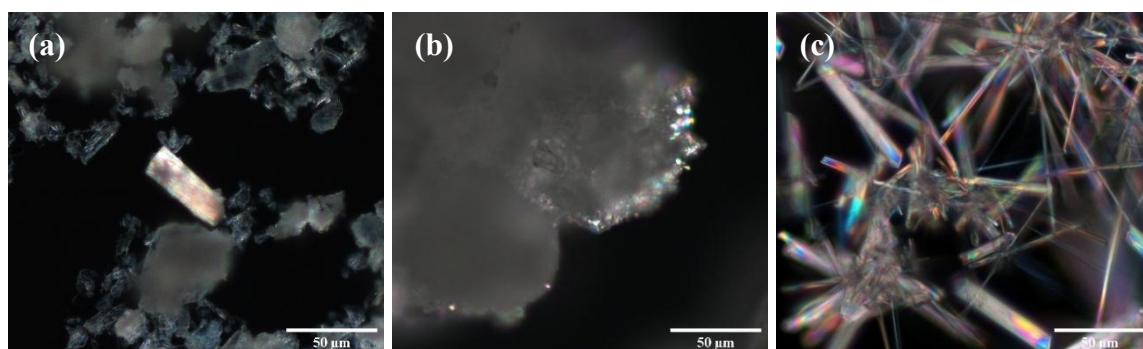


**Figure 4.7:** (a) PLM image of the birefringence of a VAL crystal in as bought (pure) valsartan at 20x magnification. (b) PLM image of NIC particles at 20x magnification. (c) PLM image of NIC crystals recrystallised in the presence of water at 20x magnification.

Figure 4.7 (a), (b) and (c) are the polarised light images of the pure components, VAL, and NIC at 20 times magnification, respectively. Figure 4.7 (a) is an image of the as-received valsartan powder, placed on a glass slide. Birefringence is observed in some isolated, irregular faceted particles, indicating the presence of some crystallinity in as-

received VAL. [Figure 4.7 \(c\)](#) demonstrates the crystallinity of NIC, which exists in the form of needles upon recrystallisation in water. NIC crystals were studied after recrystallisation because the excipient is highly unstable in an amorphous state, and only exists in clusters of crystals, where birefringence was not observed in the centre of the particle but at the edges, in the excipient's dry state due to apparent isotropy, where the clustering is not as intense as observed in [Figure 4.7 \(b\)](#).

[Figure 4.8](#) shows solid-state, dry particles of VAL:NIC co-amorphous systems of varying molar ratios at 10x magnification, starting with systems with higher amounts of VAL with respect to NIC (with the ratio 3.50:1) through to the system with higher amounts of NIC, where the VAL:NIC ratio is 1:2. The amorphous particles are observed as irregular structures, with birefringence within them. Birefringence in the form of bright, multi-coloured needles can be assigned to NIC, based on the PLM image of the component in [Figure 4.7 \(b and c\)](#). With increasing amounts of NIC, NIC crystallites became more prominent within the CAS systems. The systems with higher amount of VAL did not observe any crystallites in the form of needle-like crystals however birefringence was observed in the particles. For instance, in the VAL:NIC ratio 1.25:1, birefringence was observed in a circular morphology, vastly different from that of NIC crystalline needles (255). This difference in the crystal morphology suggests that this crystallinity arises from VAL. Between the CASs 3.50:1 to 1.25:1, 10 images of each of the systems were taken with multiple particles present in each of the images. Such birefringence was observed in all the images, indicating the presence of VAL crystallites in the systems that was not detected by XRD. As the amount of VAL in the systems decreases, the population of the particles with such birefringence goes down; it slowly reduces as the equimolar ratio approaches.

From the 2:1 VAL:NIC CASs, needle-like crystals start to appear, along with VAL crystals, and the occurrence of the needles slowly increases as the amount of NIC starts to increase with respect to VAL. Initially, 2 out of 10 images were able to detect small NIC crystals, with the crystals being present in low amounts (one or two needles observed in an image with multiple particles). In the CASs 1.75:1 and 1.50:1, the presence of needle-like crystals is observed in 9 out of 10 images. This trend slightly changes in the CAS 1.25:1, where the presence of NIC crystallinity has reduced, being present only in 4 out of 10 images. However, VAL crystallinity is prominent in this system and appears to be present in particles without the presence of NIC crystals. This suggests that within

the amorphous particles, some of the VAL did not bond with NIC and may have retained some VAL crystallinity, suggesting the presence of inhomogeneity within the system.

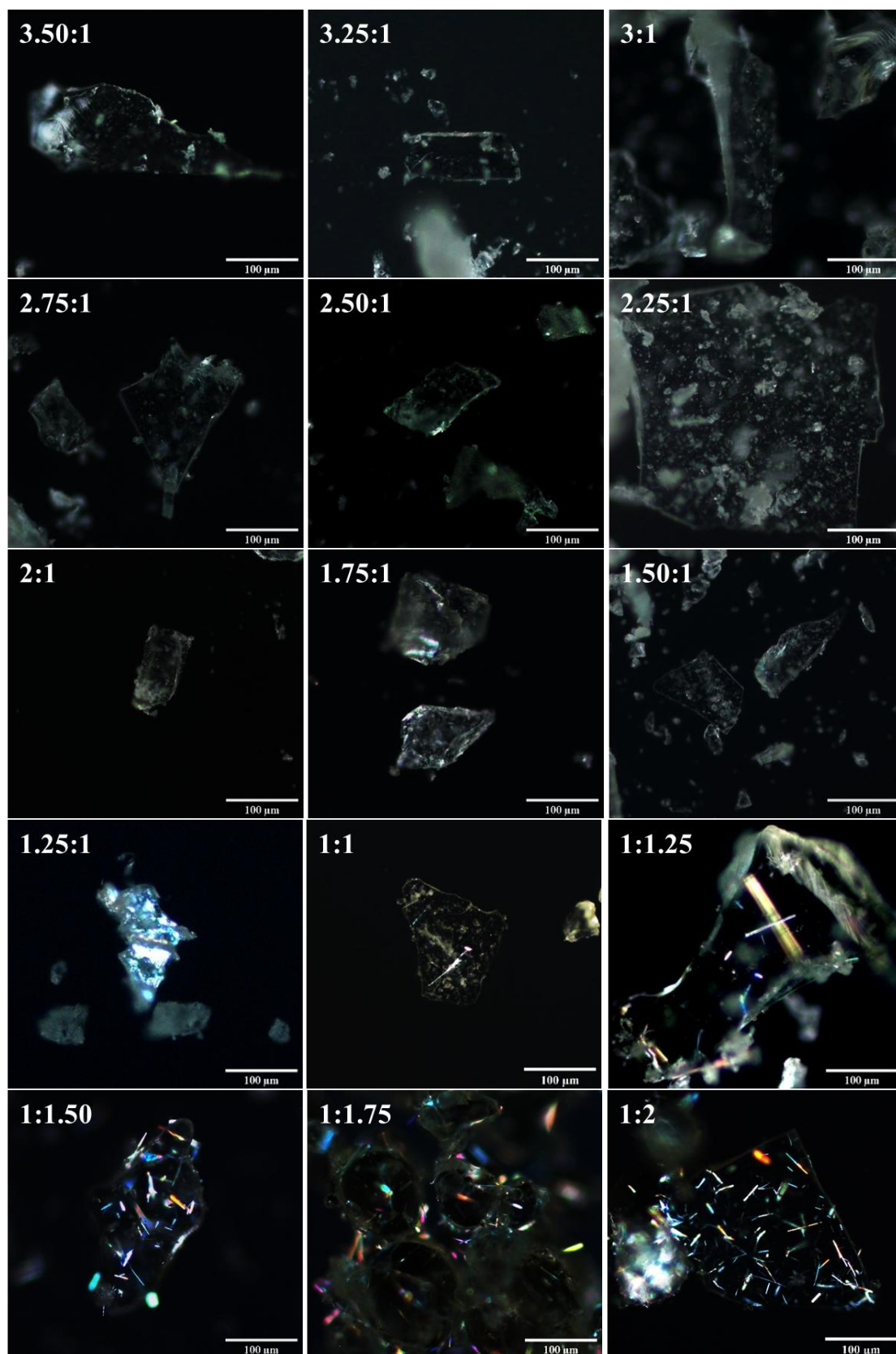


**Figure 4.8:** PLM images of VAL:NIC MA CASs from ratios 3.50:1 to 1:2, as labelled in the images at 10x magnification.

In the CASs with higher amounts of NIC, i.e., 1:1.25, 1:50, 1:1.75 and 1:2 systems, a very high quantity of NIC crystallites in the particles was observed in all the images taken, with the presence of NIC crystallites increasing with increasing amount of the excipient, consistent with the molar ratios. VAL crystallinity is harder to detect in these systems, due to the overwhelming quantities of NIC crystals.

In the 1:1 equimolar system, NIC crystallinity is present in all the images. However, the number of needles present in the particles is significantly lower than the systems with higher NIC content. Simultaneously, birefringence due to VAL is also lower in these particles. Based on the lack of excess of crystallinity from either of these components, it can be said that the equimolar system is the most optimal with the least amount of retained crystallinity from both components and potentially, the most homogeneous ratio out of all the 15 systems.

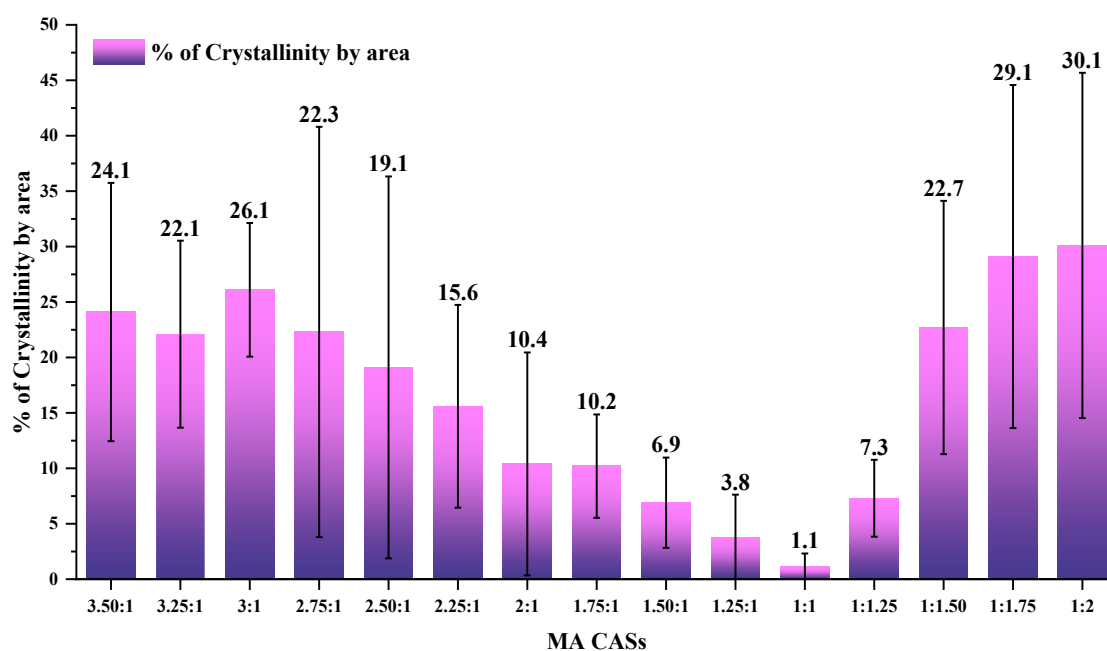


**Figure 4.9:** Trend in crystallinity by area in the MA CASs of VAL:NIC with molar ratios of 3.50:1 to 1:2, obtained by analysing 10 images of each sample (with a standard deviation depicted by the error bars). The equimolar system has the least crystallinity across the compositional space. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size.

To understand the overall crystallinity within the CASs across the compositional space, the retained crystallinity of the systems was quantified by evaluating the percentage of crystallinity in the samples with respect to area. 10 images of each of the

samples were used to study their total crystallinity and has been presented in [Figure 4.9](#). Crystallinity (per area) increased with increasing amounts of either of the components and reaches a minimum of 1.1% when it reaches the equimolar system, confirming that the 1:1 system has the least retained crystallinity. The CAS with the highest VAL content showed 24.1% of crystallinity and the system with the highest NIC content showed the highest crystallinity of 30.1% across the compositional space. This high crystalline content can be attributed to the high recrystallisation tendency of NIC (149, 251).

This sheds light on the crystallinity of the systems. Despite the pXRD patterns demonstrating that all these systems are X-Ray amorphous further investigation via PLM demonstrated that there is crystallinity from both the components in all the systems, which is otherwise undetectable via pXRD.

#### 4.3.4. Computational Studies

To understand the intermolecular interactions within the CAS, three supramolecular heterosynthons were designed in GaussView 6.1.1 and computational FT-IR spectra were calculated with the hybrid functional B3LYP combined with the 6-311++G(d,p) basis set within Gaussian 09 and GaussView, based on the work of Turek *et al* (10). The first two heterosynthons, which were identified by Turek *et al*, namely VAL-NIC1 and VAL-NIC2 were designed in an equimolar fashion, i.e., one molecule of valsartan was bonded to one molecule of nicotinamide, with both these heterodimers possessing the lowest energies obtained by optimisation with the semiempirical AM1 method (10). The VAL-NIC1 heterosynthon has two hydrogen bonds between the acid group of VAL and amide group of NIC, i.e.,  $\text{N-H}_{\text{NIC-amide}} \cdots \text{O}_{\text{VAL}}$  and  $\text{O-H} \cdots \text{O}_{\text{NIC-amide}}$  heterosynthon, as shown in [Figure 4.10 \(a\)](#). The VAL-NIC2 system also has two hydrogen bonds, one connecting the tetrazolyl H of VAL to the amide O of NIC and the other connecting the amide O of VAL to the N-H bond of NIC, i.e., tetrazolyl-amide-amide –  $\text{N-H}_{\text{VAL-tetrazolyl}} \cdots \text{O}_{\text{NIC-amide}}$  and  $\text{N-H}_{\text{NIC-amide}} \cdots \text{O}_{\text{VAL-amide}}$  heterosynthon, as presented in [Figure 4.10 \(b\)](#).

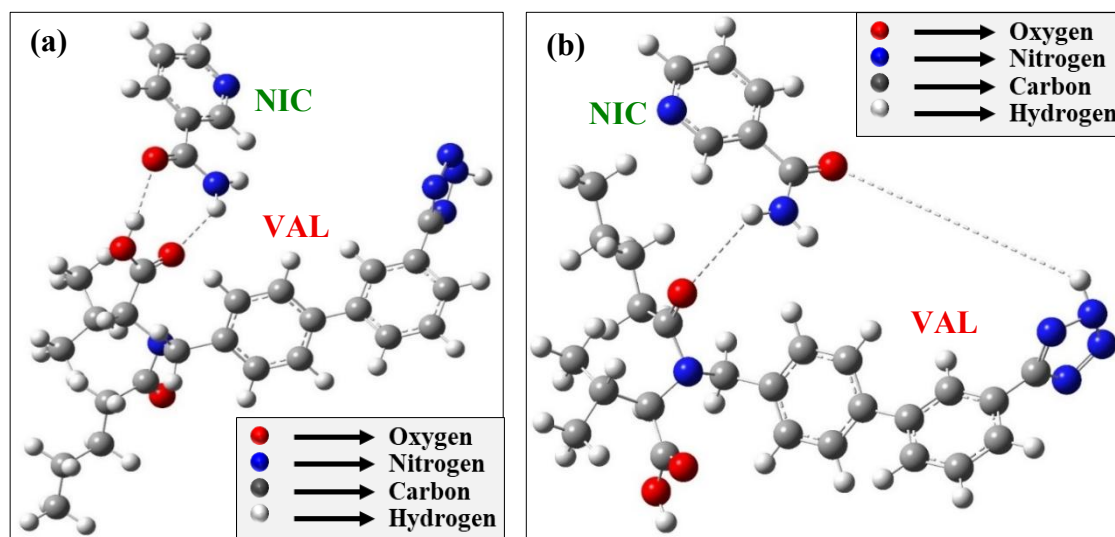


**Figure 4.10:** (a) VAL-NIC1 (acid-amide – N-H<sub>NIC-amide</sub>---O<sub>VAL</sub> and O-H---O<sub>NIC-amide</sub>) heterosynthon. (b) VAL-NIC2 (tetrazolyl-amide-amide – N-H<sub>VAL-tetrazolyl</sub>--O<sub>NIC-amide</sub> and N-H<sub>NIC-amide</sub>---O<sub>VAL-amide</sub>) heterosynthon. Modelled and optimised in GaussView 6.1.1.

Based on the [Figure 4.10 \(b\)](#), it appears that the (C=O) of NIC is quite the distance away from the tetrazolyl H of VAL, which suggests that the hydrogen bonding interaction between these two groups is weak. Additionally, this N-H<sub>VAL-tetrazolyl</sub>--O<sub>NIC-amide</sub> bond does not form a linear arrangement between the hydrogen bond donor and acceptor molecules, having a bond angle of 142°, with the tetrazolyl-H of VAL perturbed out of the plane. A direct alignment (almost 180°) would make way for a stronger and more stable bond as it puts the positive and negative charges as close as possible which leads to better overlap of orbitals, and such an alignment is not observed in the VAL-NIC2 heterosynthon. This non-linear arrangement weakens the bond holding the atoms together, leading to a geometrically strained system which is far from being a state having the lowest energy. These suggests that the VAL-NIC2 system theoretically may not be a viable heterodimer.

Given the huge size of VAL molecule in comparison to the excipient NIC, it was possible to model a 1:2 (VAL:NIC) three-molecule heterosynthon due to the multiple bonding sites of the drug molecule. The third heterosynthon was designed in a manner where both the equimolar heterosynthons come together to form a system where the large VAL molecule is able to bond with two NIC molecules, in the manner of the binary heterosynthons, as presented in [Figure 4.11](#).

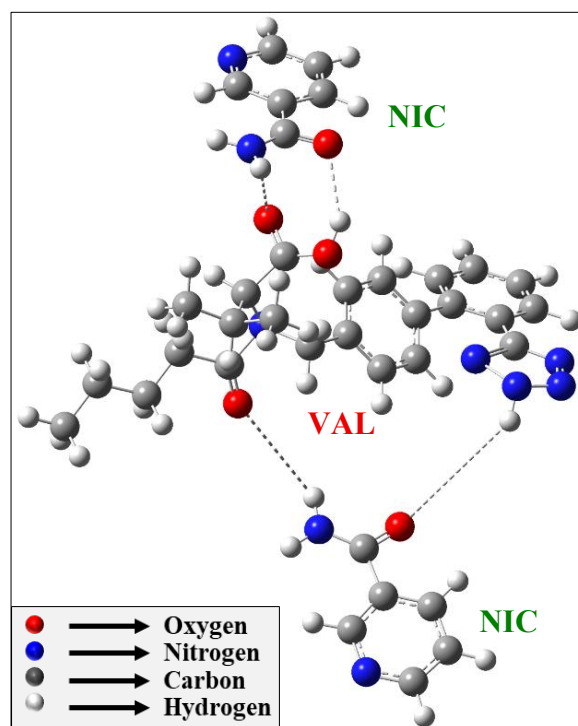


**Figure 4.11:** NIC-VAL-NIC system is a combination of the two idealised conformation, namely VAL-NIC1 (acid-amide –  $\text{N-H}_{\text{NIC-amide}} \cdots \text{O}_{\text{VAL}}$  and  $\text{O-H} \cdots \text{O}_{\text{NIC-amide}}$ ) and VAL-NIC2 (tetrazolyl-amide-amide –  $\text{N-VAL-tetrazolyl} \cdots \text{O}_{\text{NIC-amide}}$  and  $\text{N-H}_{\text{NIC-amide}} \cdots \text{O}_{\text{VAL-amide}}$ ) heterosynthons. Modelled and optimised in GaussView 6.1.1.

Geometry optimisation was performed in all three systems by semi-empirical AM1 method prior to the DFT calculations of the IR spectra. The DFT calculation produced IR data for the equimolar binary system and the three-molecule heterosynthon, demonstrating the possibility of such a system. The IR spectra are explored more in section 4.3.5.

### 4.3.5. Infrared Spectroscopy

#### 4.3.5.1. FT-IR Spectroscopy

FT-IR spectroscopy has been employed on the pure components and the CASs to study the component interaction within the CASs of varying ratio by comparing to the spectra of the pure components.

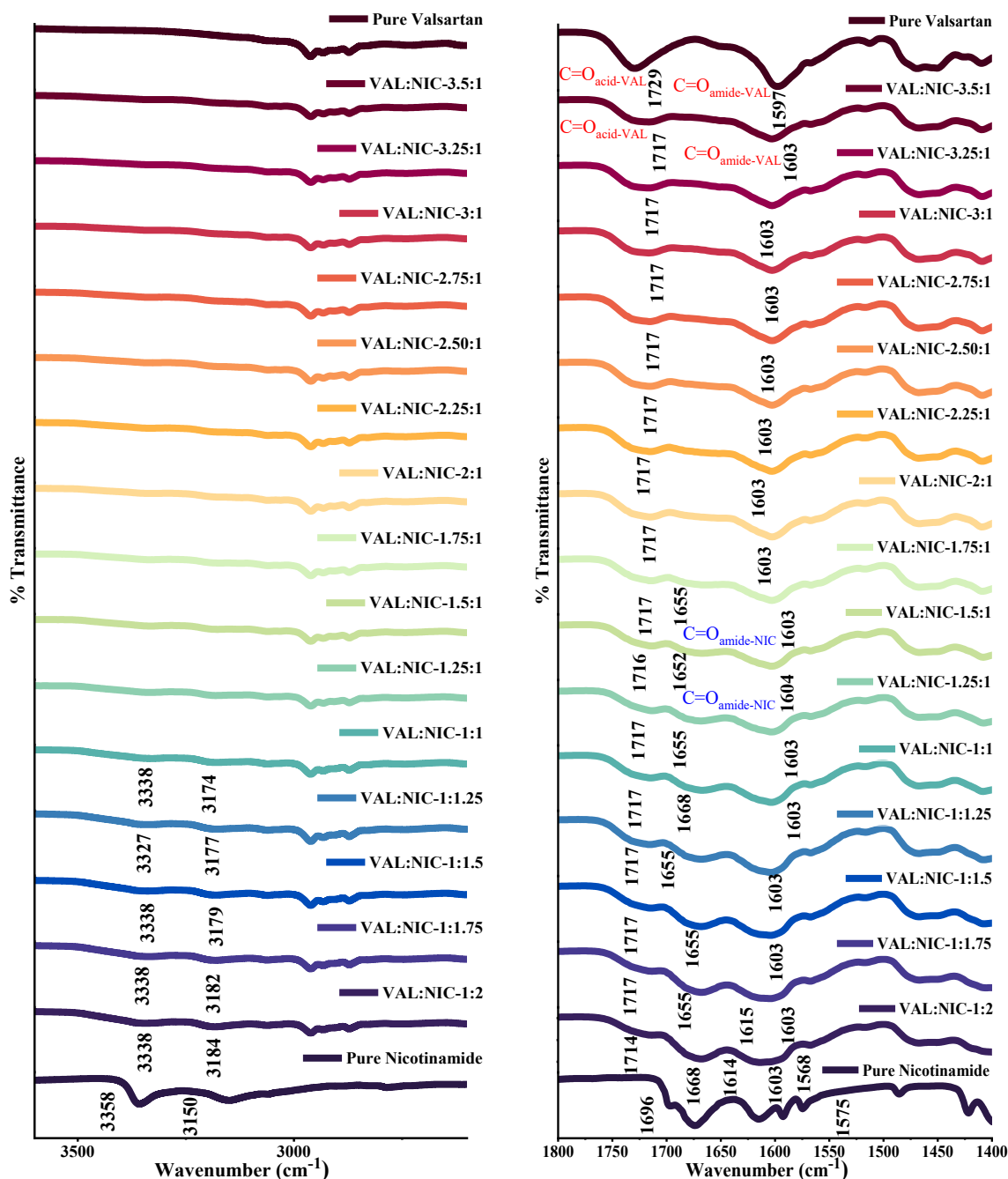


**Figure 4.12:** FTIR spectra of co-amorphous VAL-NIC systems, at different molar ratios, from 3.50:1 to 1:2. FTIR spectra of VAL and NIC are also included in the figure.

The FT-IR spectra of VAL, NIC, and the co-amorphous systems with varying ratios (VAL:NIC ratios from 3.50 to 1:2.), are presented in [Figure 4.12](#). The peaks in the amorphous CAS FT-IR spectra are broader than that of the original materials, a common occurrence in amorphous form products (142, 256). Potentially, four types of hydrogen bonds can exist in the CAS between VAL and NIC. They can form between the amide group of NIC and acid group of VAL, as in the case of VAL-NIC1 heterodimer, demonstrated in [Figure 4.10 \(a\)](#). The other two hydrogen bonds can form between the

amide group of NIC and the tetrazolyl H of VAL and carbonyl group of VAL, as demonstrated in [Figure 4.10 \(b\)](#). The FT-IR spectrum of VAL shows two characteristic sharp peaks at  $1729\text{ cm}^{-1}$  and  $1597\text{ cm}^{-1}$ , corresponding to the carbonyl (C=O) in acid and (C=O) in amide stretching vibrations, respectively. The broad peak with maxima at  $3120\text{ cm}^{-1}$  can be assigned to the OH stretching vibration of VAL. A weak absorption band at  $3455\text{ cm}^{-1}$  for NH tetrazole has been observed in the VAL spectrum. The FT-IR spectrum of NIC shows two characteristic peaks at  $3358\text{ cm}^{-1}$  and  $3150\text{ cm}^{-1}$ , which correspond to the asymmetric and symmetric stretching vibrations of  $\text{NH}_2$  groups,  $1674\text{ cm}^{-1}$  (with a shoulder at  $1696\text{ cm}^{-1}$ ) corresponds to the stretching vibration of carbonyl (C=O) and peaks at  $1615\text{ cm}^{-1}$  and  $1575\text{ cm}^{-1}$  correspond to the bending vibration of NH. The intensity of C=O of NIC is much stronger than C=O in acid of VAL but lower than that of C=O in amide of VAL.

The shifting of wavenumbers of peaks in the functional groups which form hydrogen bonds between the components in an equimolar system are presented in [Table 4.2](#). A comparative analysis of the absorption bands of the H-bond forming functional groups of the starting compounds with that of the CASs shows clear shifting patterns of the peaks, which suggests the formation of intermolecular H-bonds between the two components (10). The OH stretching peak of VAL has shifted to a higher value of  $3174\text{ cm}^{-1}$  from  $3116\text{ cm}^{-1}$ , which can be due to the decrease in bond length in the OH bond, as the H potentially bonded to the amide O of NIC and forms a O-H-O synthon, while the (C=O) peak of NIC has shifted to a lower value, from  $1674\text{ cm}^{-1}$  to  $1653\text{ cm}^{-1}$ , possibly due to a bond lengthening due to this hydrogen bond. Furthermore, both the NH stretching vibrations have shifted to lower values, from  $3358\text{ cm}^{-1}$  and  $3150\text{ cm}^{-1}$  to  $3174\text{ cm}^{-1}$  and  $3060\text{ cm}^{-1}$  respectively, potentially due to the acid O of VAL, forming a N-H-O synthon with the NH of NIC, which too shows lowering of stretching frequency, from  $1729$  to  $1717\text{ cm}^{-1}$ . This downward shift was also observed by the NH bending vibrations due to this hydrogen bonding, from  $1615\text{ cm}^{-1}$  and  $1575\text{ cm}^{-1}$  to  $1603\text{ cm}^{-1}$  and  $1567\text{ cm}^{-1}$ . Both these bonds are presented in the VAL-NIC1 heterosynthon with  $\text{N-H}_{\text{NIC-amide}}\cdots\text{O}_{\text{VAL}}$  and  $\text{O-H}\cdots\text{O}_{\text{NIC-amide}}$  bonding in the system, as demonstrated in [Figure 4.10 \(a\)](#). However, the peak assigned to asymmetric stretching vibration of the NH bond of NIC, remains partially unshifted and stays at  $3358\text{ cm}^{-1}$ , which indicates that only one NH bond binds to the  $\text{C=O}_{\text{acid}}$  group at a time, whilst the other one exists freely. This unbonded NH of NIC bond can then bond with another molecule of VAL which can lead to an excess of unbonded NIC and cause phase separation. Furthermore, it can bond with water

molecules present due to moisture in the surroundings which could potentially disrupt the stability of the co-amorphous system.

The  $\text{C=O}_{\text{amide}}$  of VAL undergoes a small increase of  $6\text{ cm}^{-1}$  to  $1603\text{ cm}^{-1}$  (merged with the NH bending vibrations), possibly due to the hydrogen bonding interactions with the NH of NIC. Additionally, the tetrazolyl NH peak of VAL at  $3455\text{ cm}^{-1}$  goes undetected across all the all the molar ratios, which indicates that it has undergone hydrogen bonding interactions with the  $\text{C=O}_{\text{amide}}$  of NIC, as indicated by its red shift. Both of these bonding are present in VAL-NIC2 heterosynthon in [Figure 4.10 \(b\)](#).

Systems with lower amounts of NIC appear to have the NH stretching vibrations missing up to the system with a VAL:NIC ratio of 1.25:1. Based on the [Figure 4.12](#), the shifts for the CASs with higher VAL content (3.50:1 to 1.25:1) reflect the same peak shifts as the equimolar system, however the peaks pertaining to VAL are much more intense than the 1:1 system, as the peak intensity of the characteristic absorbance increases with increasing concentration (222). The same trend of peak shift indicates that there is hydrogen bonding between the two components. However, the presence of such strong peaks could be indicative of a lack of uniform hydrogen bonding between the two components, which is indicative of inhomogeneous bonding and can potentially lead to unbonded VAL within these systems, resulting in phase separation through the system. As these amorphous systems are hygroscopic by nature, these unbonded sites can easily bond with the surrounding moisture and disrupt the stability of these systems further.

Adding more amount of NIC to the co-amorphous system may not be a solution either. Just like in the systems with excess VAL, the vibrational peaks of the functional groups of NIC get broader and become more intense and show the same peak shifts as the equimolar system. It has been difficult to understand the hydrogen bonding interaction between the two components as the stretching vibrations of the NH groups in NIC gets more and more intense, and it is difficult to observe any significant change in their positions as it had done in the equimolar system (see [Table 4.2](#)) It seems that any hydrogen bonding in these high NIC content CASs is overshadowed by the amount of freely existing, unbonded NIC within the systems.

**Table 4.2:** Experimental Values of FT-IR spectra for VAL, NIC, and melt amorphised co-amorphous systems along with shifts ( $\Delta$ ) relative to VAL and NIC. The functional groups highlighted in blue are potentially involved in hydrogen bonding.

| <u>Functional Groups</u>            | <u>Frequency (cm<sup>-1</sup>)</u> |                         |                                         |
|-------------------------------------|------------------------------------|-------------------------|-----------------------------------------|
|                                     | <u>VAL</u>                         | <u>NIC</u>              | <u>MA CAS MA ratios 3.25:1 to 1:2)</u>  |
| (O-H) stretching                    | 3116                               | -                       | 3174 ( $\Delta=+59$ )                   |
| $\nu(\text{NH})_{\text{tetrazole}}$ | 3455 (poor signal)                 | -                       | -                                       |
| Acid (C=O) stretching               | 1729                               | -                       | 1717 ( $\Delta=-12$ )                   |
| Amide (C=O) stretching              | 1597                               | -                       | 1603 (Shoulder at 1612) ( $\Delta=-6$ ) |
| $\nu(\text{C-O})$                   | 1390                               | -                       | 1389 ( $\Delta=-1$ )                    |
| Unreacted (N-H) as. stretching      | -                                  | 3358                    | 3358                                    |
| (N-H) as. stretching                | -                                  | 3358                    | 3174 ( $\Delta=-184$ )                  |
| (N-H) stretching                    | -                                  | 3150                    | 3060 ( $\Delta=-90$ )                   |
| (C=O) stretching                    | -                                  | 1674 (shoulder at 1696) | 1653 ( $\Delta=-21$ )                   |
| (N-H) bending                       | -                                  | 1615, 1575              | 1603, 1567 ( $\Delta=-12, -8$ )         |
| $\nu(\text{C-N})$                   | -                                  | 1340                    | 1269 ( $\Delta=-71$ )                   |
| $\rho\omega(\text{NH}_2)$           | -                                  | 777                     | 777 ( $\Delta=0$ )                      |

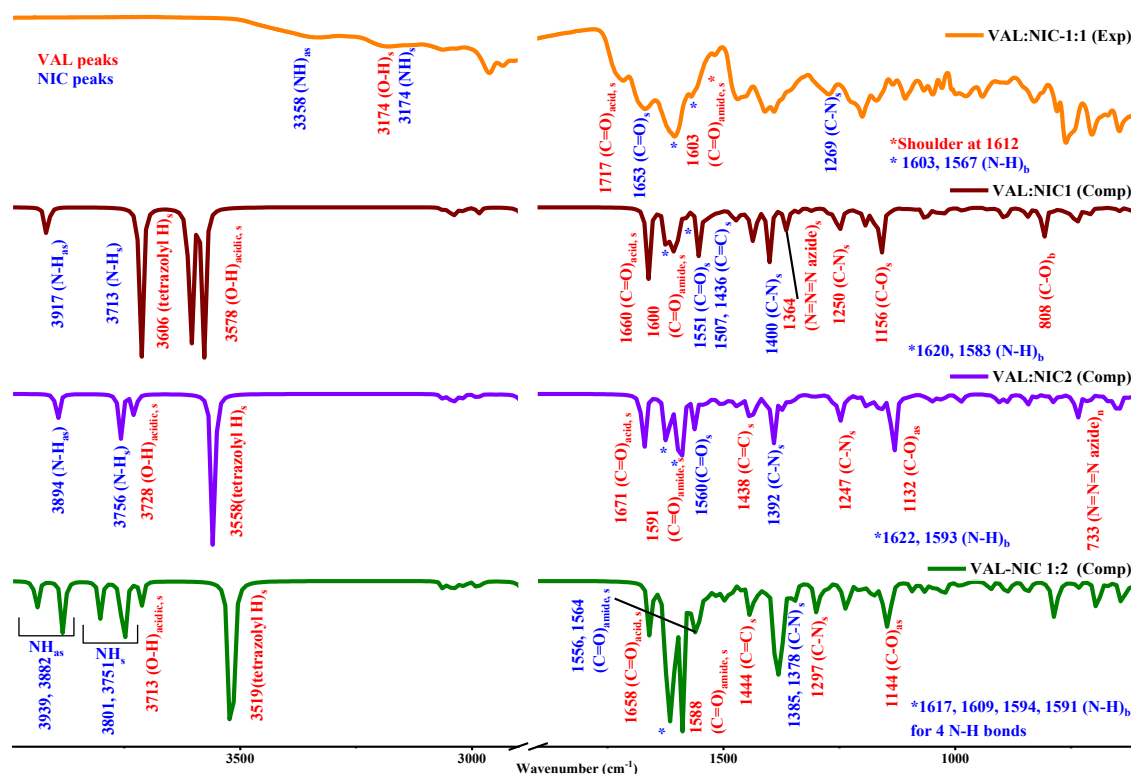


**Figure 4.13:** (In orange, red and purple) Experimental and computational FT-IR spectra for equimolar co-amorphous system of valsartan and nicotinamide. In green, computational FT-IR spectrum for VAL:NIC 1:2 systems.

The computational IR spectra by DFT calculation from the two binary heterodimers VAL-NIC1 and VAL-NIC2 and the three-molecule heterosynthons described in section 4.3.4, were then compared to that of the experimental spectra equimolar of co-amorphous system, as shown in Figure 4.13. The computed IR of the bimolecular heterosynthons appear to be different to the experimental spectrum of the equimolar CAS with respect to peak position, intensities, and width, in the 4000 – 3500  $\text{cm}^{-1}$  range. The N-H stretching vibrations of NIC has increased to higher wavenumbers in the calculated spectra, from 3358  $\text{cm}^{-1}$  and 3150  $\text{cm}^{-1}$  to 3894  $\text{cm}^{-1}$  and 3756  $\text{cm}^{-1}$ , almost by a factor more than 500, which is contradictory to what we observe in the experimental data. Most often, DFT calculations of a molecule produce results that are close to its experimental infrared spectrum. The DFT method for this study (B3LYP) is not effective in describing long range interactions, unless "corrections" are made, i.e., the methods should have a proper basis set such as 6-31++G (d,p), as used in this study (237). However, in hydrogen bonding of the X-H (X=O, N) functional groups, the system might exhibit some anharmonicity; such an approximation may or may not have considered in the DFT calculations, which could potentially lead to a shift in peaks (237, 257).

However, the C=O stretching frequencies follow the opposite trend, exhibiting a red shift (237, 257). The calculated spectra shift C=O<sub>acid</sub> peak of VAL to a lower wavelength of 1660 cm<sup>-1</sup> (VAL-NIC1) and 1671 cm<sup>-1</sup> (VAL-NIC2) from the experimental value of 1717 cm<sup>-1</sup>. Additionally, the C=O<sub>amide</sub> stretching frequencies for both VAL and NIC show a downward shift in the peak intensities in their calculated values, from 1603 cm<sup>-1</sup> to 1600 cm<sup>-1</sup> (VAL-NIC1) and 1591 cm<sup>-1</sup> (VAL-NIC2) for VAL and from 1653 cm<sup>-1</sup> to 1551 cm<sup>-1</sup> (VAL-NIC1) and 1560 cm<sup>-1</sup> (VAL-NIC2) for NIC. Furthermore, the calculated peaks differ in intensities with respect to the experimental data.

The calculated spectrum of the third heterosynthon seems to be a better fit to the experimental data. Due to the presence of three C=O stretching vibrations and amorphisation, peak broadening and overlapping is expected (256). Although this tri-molecular heterosynthon shows the same trend as the calculated binary heterosynths with respect to the N-H and C=O stretching vibrations, it is the C=O stretching vibrations that makes the most impact. The experimental C=O peaks at 1717 cm<sup>-1</sup>, 1653 cm<sup>-1</sup> and 1603 cm<sup>-1</sup> are a closer match to peaks at 1658 cm<sup>-1</sup>, 1588 cm<sup>-1</sup> and 1564 cm<sup>-1</sup> of the IR data of the third heterosynthon due to similarities in intensities and shape, than that of the heterodimers VAL-NIC1 and VAL-NIC2. Due to this overlapping of the C=O signals as well as the similarity between the experimental spectra and the third heterosynthon within the range of 1700 to 1500 cm<sup>-1</sup>, it can very well be a case where more than one molecule of VAL may bond with two molecules of NIC, existing in an equimolar co-amorphous system, potentially leaving an excess of unbonded amorphous VAL, thus creating an inhomogeneity within such a system.

#### 4.3.5.2. Raman Spectroscopy

Raman spectroscopy has been employed to study *additional interaction* within the co-amorphous systems, that the FT-IR does not readily detect. The inVia™ Renishaw equipment can measure between 3200 to 400 cm<sup>-1</sup>. However, the interaction involving carbonyl groups can be observed between the range of 1800 to 1500 cm<sup>-1</sup>, as presented in [Figure 4.14](#). VAL shows three characteristic peaks at 1608 cm<sup>-1</sup>, 1583 cm<sup>-1</sup> and 1564 cm<sup>-1</sup>, corresponding to C=O<sub>amide</sub> stretching, C=C stretching and NH bending vibrations, respectively. The NIC showed characteristic peaks at 1580 cm<sup>-1</sup> which corresponding to the C=C stretching, whilst 1598 cm<sup>-1</sup>, 1616 cm<sup>-1</sup>, and 1679 cm<sup>-1</sup>, where the first two

correspond to the NH bending vibrations and the last peak corresponds to the C=O stretching vibrations.

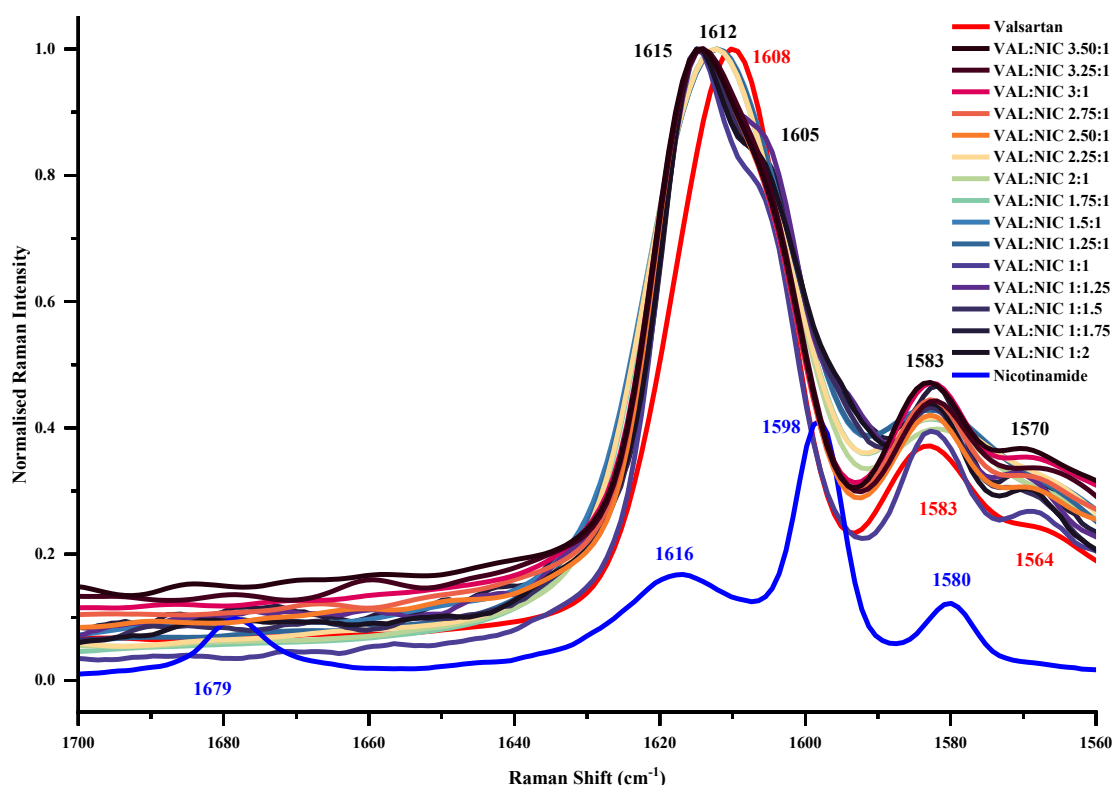


**Figure 4.14:** Normalised Raman spectra of valsartan, nicotinamide, and melt amorphised co-amorphous systems. Spectra are shown between the ranges of 1700 to 1560  $\text{cm}^{-1}$ , with the background removed.

A change in the peak positions is observed in the CASs. Potentially, the amide carbonyl group of nicotinamide is involved in an intermolecular hydrogen bond. The  $\text{C=O}_{\text{NIC}}$  peak cannot be detected in any of the CASs (232). In an amide-acid heterosynthon, there is an increase in the amide carbonyl bond length (in NIC) as the carbonyl oxygen forms a hydrogen bond with VAL. The peak at  $1679 \text{ cm}^{-1}$  decreases till it merges with the background as in Figure 4.14.  $\text{C=O}_{\text{amide-VAL}}$  stretching frequency increases to  $1615 \text{ cm}^{-1}$  from  $1608 \text{ cm}^{-1}$  (sometimes at  $1612 \text{ cm}^{-1}$ ) and the small peak at  $1564 \text{ cm}^{-1}$  can be assigned to  $\text{N-H}_{\text{tetrazolyl}}$  bending, which increases to  $1570 \text{ cm}^{-1}$  in the CASs. These shifts could be due the second bonding site which involves N-H of NIC bonding with  $\text{C=O}_{\text{amide}}$  of VAL and  $\text{C=O}_{\text{amide}}$  of NIC which bonds with  $\text{N-H}_{\text{tetrazolyl}}$  of VAL, a bonding that occurs in VAL-NIC2 heterosynthon as shown in the Figure 4.10 (b). Due to the rigidity of the VAL molecule in this heterosynthon, the decrease in bond length is low, thus the shift is lower.

**Table 4.3:** Experimental Values of Raman spectra for VAL, NIC, and melt amorphised co-amorphous systems along with shifts ( $\Delta$ ) relative to VAL and NIC. Stretching and bending interactions are demoted by the suffices s and b respectively and UD indicates that the peaks go undetected.

| Functional Groups        | VAL  | NIC           | CAS (MA ratios 3.25:1 to 1:2)                |                         |
|--------------------------|------|---------------|----------------------------------------------|-------------------------|
|                          |      |               | VAL                                          | NIC                     |
| C=O (amide) <sub>s</sub> | 1608 | 1679          | 1615 ( $\Delta=7$ ),<br>sometimes at<br>1612 | UD                      |
| N-H <sub>b</sub>         | 1564 | 1616,<br>1598 | 1570 ( $\Delta=6$ )                          | UD, 1606 ( $\Delta=8$ ) |
| C=C <sub>s</sub>         | 1583 | 1580          | 1570 ( $\Delta=-13$ )                        | 1570 ( $\Delta=-10$ )   |

The peak shifting from  $1598\text{ cm}^{-1}$  of crystalline NIC to  $1606\text{ cm}^{-1}$  (a shoulder) in the amorphous form, was assigned to the vibration of the N-H bond (increase in bond length due the H-bonding, which increases the electron density at the N, causing an increase in bond angle, thus increasing bending vibration), a shift similar to that of the N-H<sub>tetrazolyl</sub> bending vibration of VAL. The peak at  $1616\text{ cm}^{-1}$  merges with the background. This poor signal of the N-H vibrations is likely due to the high polarizability of the heteronuclear N-H bonds, that generally produces weak signals in Raman spectroscopy, although strong signals in FT-IR spectroscopy, as evidenced by the data in section 4.3.5.1. This suggests that the shift was related to the formation of hydrogen bonds between the two molecules. In an amide-acid heterosynthon, there is an increase in the amide carbonyl bond length (in NIC) as the carbonyl oxygen forms a H-bond with the hydroxyl H of VAL, thus increasing the C=O bond length of NIC, thus causing a lowering of frequency, making the peak undetectable in the co-amorphous systems. The peak shifts are presented in Table 4.3. The Raman data show that the bonding occurs in VAL-NIC<sub>2</sub> form, where N-H<sub>VAL-tetrazolyl</sub>--O<sub>NIC-amide</sub> and N-H<sub>NIC-amide</sub>---O<sub>VAL-amide</sub> bonding is expected, as depicted in Figure 4.10 (b).

The downward shift in peaks corresponding to C=C stretching vibrations, from  $1583\text{ cm}^{-1}$  to  $1570\text{ cm}^{-1}$  for VAL and  $1580\text{ cm}^{-1}$  to  $1570\text{ cm}^{-1}$  for NIC, can indicate aggregation due to the formation of  $\pi$ - $\pi$  stacking interactions, between the aromatic rings

of the two components (135). This suggests that the heterosynthons are further stabilised by  $\pi$ - $\pi$  interactions, in addition to hydrogen bonding interactions.

FT-IR spectroscopy identified the polar hydrogen bonds between the acid group of VAL and amide group of NIC in the VAL-NIC1 heterosynthon present in these binary systems and detect the presence of excess of either of the components in either side of the compositional range. The presence of excess of any of the components can lead to phase separation and bonding with water molecules, either of which can hamper the stability of these CASSs. Raman spectra identified the non-polar hydrogen bonding interaction between the C=O<sub>amide</sub> group of VAL and tetrazolyl H of VAL with the amide group of NIC, thus providing additional evidence of presence of a second heterosynthon, VAL-NIC2 within the systems. Furthermore, it identified additional secondary bonding interactions, i.e.,  $\pi$ - $\pi$  stacking, which is not easy to detect just by FT-IR, that further aids in the stabilisation of the co-amorphous systems.

#### 4.3.6. Equilibrium Solubility

The main goal of formulation of co-amorphous drug delivery systems is improving the aqueous solubility of poorly soluble API (VAL). To identify which of the binary system has the better performance, the aqueous solubility of the varying co-amorphous system was tested. Equilibrium solubility of the VAL:NIC co-amorphous systems in the ratio 2:1, 1.5:1, 1:1, 1:1.5 and 1:2 along with that of VAL was assessed.

**Table 4.4:** Solubilities of co-amorphous formulations in Milli-Q water (pH 5.98) at 25 °C. Each value is an average of three repeats along with a standard deviation.

| Melt amorphised CASSs<br>(VAL:NIC) | <u>Aqueous Solubility (mg mL<sup>-1</sup>)</u> |                                           |
|------------------------------------|------------------------------------------------|-------------------------------------------|
|                                    | Binary system                                  | Solubility of VAL in the<br>binary system |
| 2:1                                | 5.72±0.30                                      | 4.85±0.03                                 |
| 1.2:1                              | 6.09±0.13                                      | 4.77±0.03                                 |
| 1:1                                | 6.05±1.15                                      | 5.04±0.15                                 |
| 1:1.5                              | 6.08±0.03                                      | 4.76±0.08                                 |
| 1:2                                | 6.17±0.04                                      | 4.6±0.01                                  |

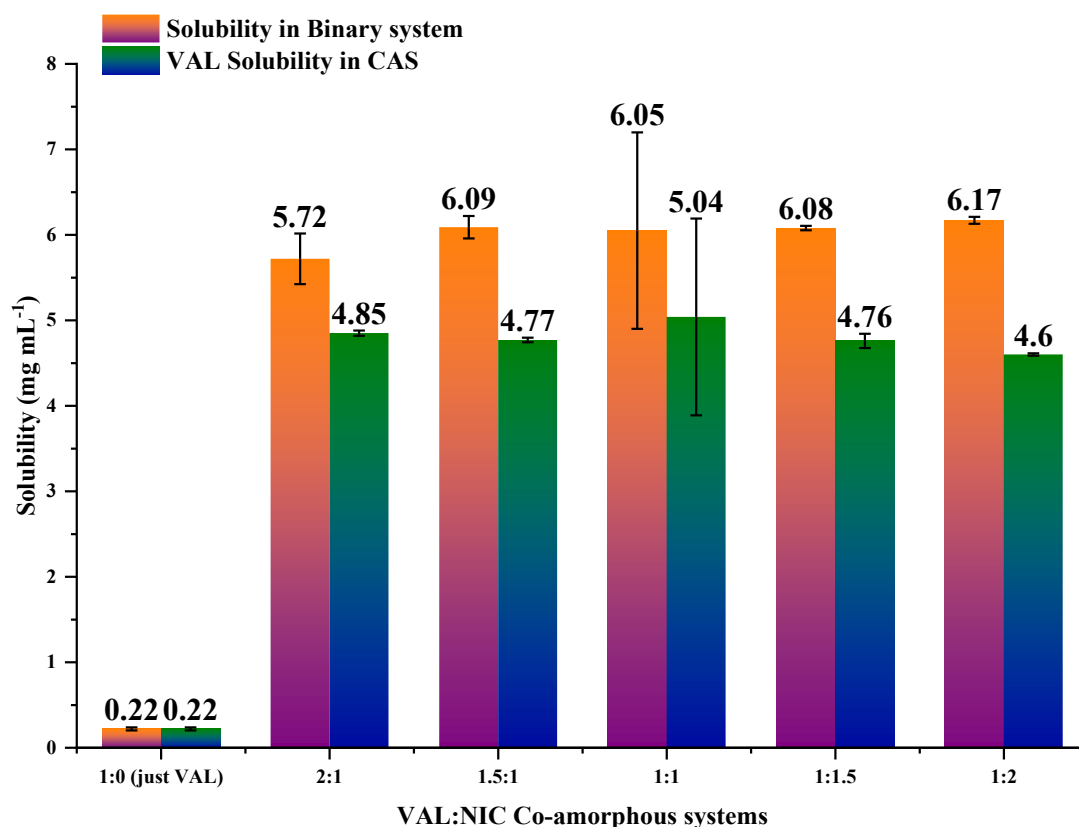


**Figure 4.15:** Equilibrium solubility values of VAL and varying ratios of co-amorphous formulations prepared by melt amorphisation in Milli-Q water (pH 5.98). The bars in purple-orange gradient represent the solubility of the CASs as a whole and the bars in green-blue gradient represent the solubility of the drug within the co-amorphous system. Each value is an average of three repeats along with error bars depicting standard deviation.

The equilibrium solubility plot for CASs is shown in [Error! Reference source not found.](#) and tabulated in [Error! Reference source not found.](#). The solubility of the VAL is 0.22 mg mL<sup>-1</sup>. However, the co-amorphisation of the drug with NIC, a highly water-soluble component with a solubility of 1 g mL<sup>-1</sup>, significantly enhanced the solubility of the formulations (258). With systems with higher VAL content, the equilibrium solubility was 5.72 mg mL<sup>-1</sup> and 6.09 mg mL<sup>-1</sup> for VAL: NIC CASs with molar ratios 2:1 and 1.5:1, respectively. For systems with higher NIC content, the equilibrium solubility was 6.08 mg mL<sup>-1</sup> and 6.17 mg mL<sup>-1</sup> for VAL:NIC CASs with molar ratios 1:1.5 and 1:2, respectively. The equilibrium solubility for the equimolar CAS is 6.05 mg mL<sup>-1</sup>. It can be observed that the equilibrium solubility of the binary system as a whole increases with increasing NIC content, as is expected due to the hydrotropic effect of NIC (31, 108). However, there is no significant differences between the CASs with varying molar ratios across the compositional space. This lack of any significant

increase in solubility in CASs with higher amounts of NIC is possibly due to the self-aggregating tendency of NIC, which reduces its efficacy towards solubility enhancement (31). Furthermore, a minimum concentration of NIC is required around VAL to enhance solubility, where the NIC molecules are present in a randomised manner (non-stoichiometric accumulation) around VAL, where the interactions between the NIC-molecules are strong enough to facilitate solubilisation but not enough to facilitate self-aggregation (31). This minimum concentration of NIC that can enhance VAL solubility the most begins at 2:1, but it might be argued that the enhancement of VAL solubility is most prominent at the equimolar composition.

Co-amorphisation enhances equilibrium solubility for the drug significantly. Across the compositional space, the solubility of the drug in the CAS has increased by 23 times with respect to the API itself. The equimolar system has the highest VAL solubility of 5.04 mg mL<sup>-1</sup> across the compositional space. This would suggest that the 1:1 system not only has the better intermolecular interaction between the drug and the excipient across the compositional space to drive solubilisation but also satisfies the minimum concentration of NIC required to demonstrate its solubilising capabilities most efficiently without undergoing self-aggregation and crystallisation (Figure 4.9), thereby improving the aqueous solubility of VAL.

#### 4.3.7. Ageing Studies

Structural changes observed by pXRD measurements and PLM images were used to predict the most recrystallisation resistant molar ratio of the co-amorphous systems when stored under high humidity conditions. Ageing studies were conducted on the VAL:NIC co-amorphous systems in the ratios of 2:1, 1.5:1, 1:1, 1:1.5, and 1:2. The selected CASs were aged in 85% relative humidity to investigate any early stages of recrystallisation. Figure 4.16 shows the pXRD patterns of the CASs before and after ageing.

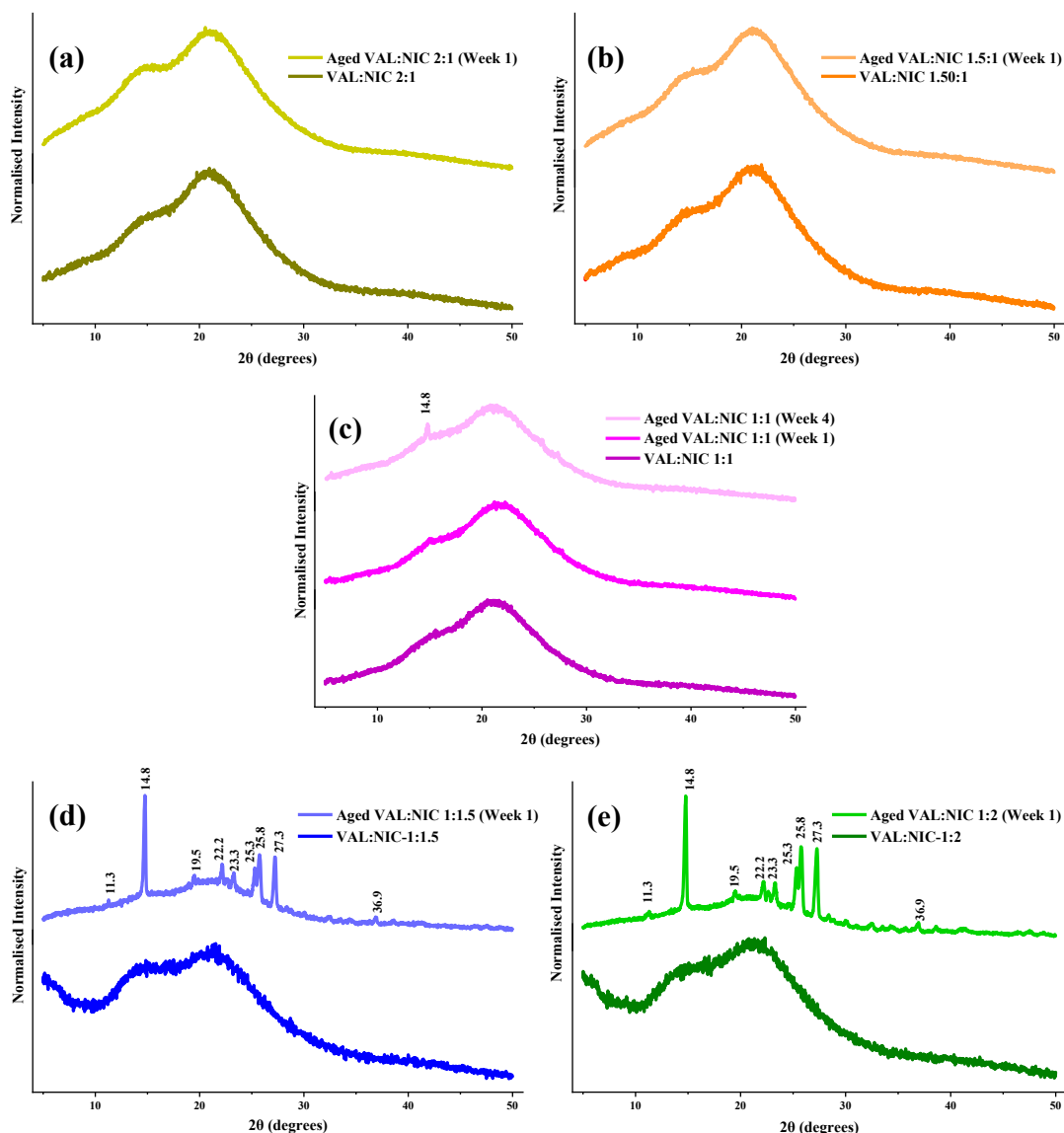


**Figure 4.16:** (a) and (b) PXR D patterns of MA CAS with excess VAL in the ratios 2:1 and 1.50:1 at freshly prepared and after one week of ageing. No recrystallisation was observed (c) PXR D pattern of equimolar VAL:NIC CAS, freshly prepared and after one and four weeks of ageing. NIC recrystallised after 4 weeks of ageing. (d) and (e) PXR D patterns of MA CAS with excess NIC in the ratios 1:1.50 and 1:2.0 at freshly prepared and after one week of ageing. Only NIC recrystallised after 1 week of ageing.

Systems with higher VAL quantities, i.e., CASs in the ratios 2:1 and 1:1.5 show no crystalline peaks after 1 week of storage, as shown in Figure 4.16 (a). Recrystallisation of equimolar MA CAS occurs at 4 weeks of ageing, as shown in Figure 4.16 (c) whilst systems with higher amounts of NIC CAS show the first signs of recrystallisation after 1 week (both by PXR D), as shown in Figure 4.16 (d) and (e). In all the systems only NIC recrystallised and no signs of VAL crystallinity was detected. It can be concluded that the VAL-rich and equimolar system are more stable than the systems with higher amounts of NIC.

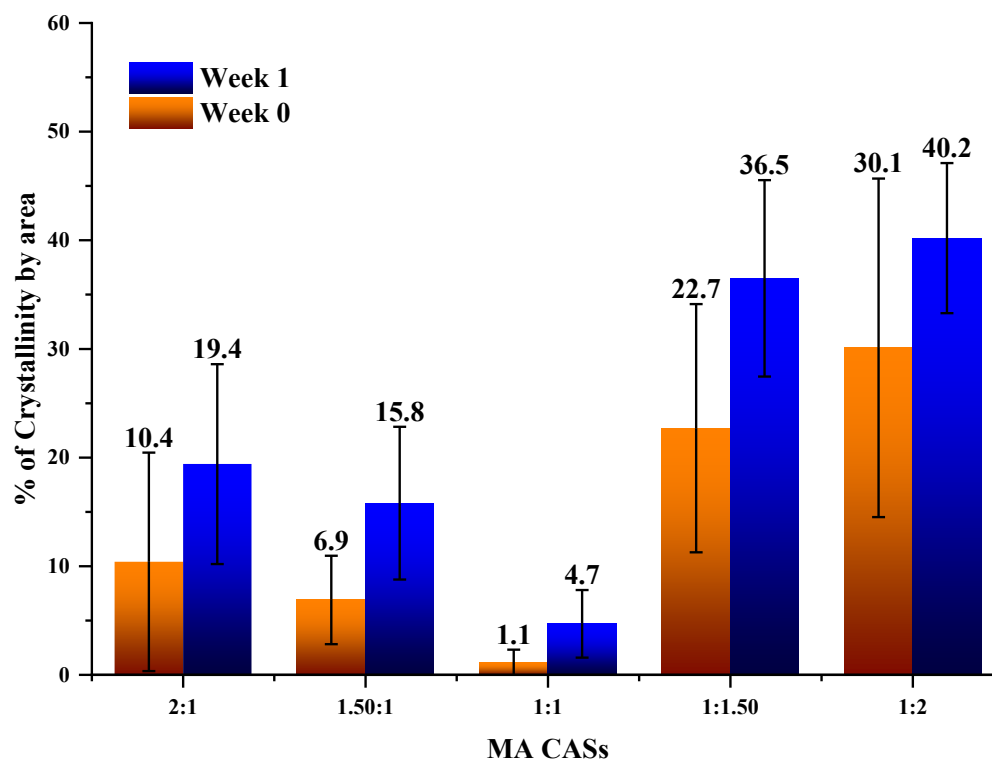


**Figure 4.17:** Crystallinity estimation after 1 week of ageing at 85% RH in VAL:NIC CASs across the compositional range (2:1, 1.5:1, 1:1, 1:1.5 and 1:2) by PLM by analysing 10 images for each sample. The equimolar system has the least increase in crystallinity across the compositional space. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size.

As observed from the pXRD data and PLM images in sections 4.3.1 and 4.3.3, pXRD relies on long-range order to produce sharp peaks and any lack thereof produces an amorphous halo, whereas PLM detects local birefringence in materials by analysing optical properties and not their atomic/molecular lattice ordering, i.e., detects crystallinity whether the material possesses long-range or small-range periodicity (259). VAL does not appear to form well-defined crystals that undergo strong constructive interference to reflect high intensity Bragg peaks i.e. low quantities may go undetected by pXRD. This undetected crystallinity is possibly due to the large size of the VAL molecule that allows it the flexibility to orient in more than one way, an arrangement that is absent in long-order crystalline forms. However, local crystallinity is easily detected by PLM. For example, PLM detected VAL crystallinity in as-prepared systems with higher VAL content (Section 4.3.3).

The 5 CASs that were subjected to ageing for 1 week at 85% RH (Figure 4.16) were analysed by percentage of crystallinity in the samples with respect to area by PLM.

They were quantified to determine changes in crystallinity due to high humidity, and the results are presented in [Figure 4.17](#). The PLM images showing the increase in crystallinity have been provided in [Appendix A](#). Crystallinity has increased significantly in systems where either of the components were in excess. For CASs with excess VAL, i.e., systems in the ratios 2:1 and 1.5:1, the crystallinity has increased from 10.4% to 19.4% and 6.9% to 15.8% respectively and those with excess NIC, i.e., systems in the ratios 1:1.5 and 1:2, the crystallinity has increased from 22.7% to 36.5% and 30.1% to 40.2% respectively. Only the equimolar CAS was the most resistant to recrystallisation, maintaining a crystallinity below 5%.

The equimolar CAS has consistently maintained the least retained crystallinity by PLM, before and after ageing, making it the optimal ratio across the compositional range.

#### 4.4. Conclusion of Chapter 4

The aim of this chapter was to identify the most appropriate composition across a wide compositional range of CASs of valsartan and nicotinamide prepared by melt amorphisation, in terms of the least retained crystallinity, closest proximity to the theoretical glass transition temperature, highest aqueous solubility and the most recrystallisation resistant when stored at high humidity, so that this optimal ratio would be used to test similar co-amorphous systems prepared by different preparation methods.

pXRD suggests that MA CASs are amorphous up to the molar ratio of 1:2 VAL:NIC; beyond that ratio, crystallinity was observed, that reflected both the drug and excipient, thus setting a limit on the amorphisation tendency of the compositional range. PLM was able to detect crystallinity in all the X-ray amorphous systems. Upon quantifying the images of the CASs across the compositional range, the equimolar system had the least retained crystallinity from both the components, thus making it the optimal ratio in terms of crystallinity.

TGA observed mass loss due to moisture increased with increasing amounts of VAL. tmDSC data from the first heating cycles suggests the presence of moisture and inhomogeneity across the compositional space in the as-prepared systems. The downward shift in  $T_g$  towards that of anhydrous NIC in systems with higher NIC content indicates the presence of amorphous, hydrated NIC. The presence of a single endothermic event in the second heating cycle suggests proper mixing of the components, along with removal of adsorbed moisture occurred in the first heating cycle. The presence of some unbonded,

crystalline NIC and unbonded, amorphous, and crystalline VAL within the equimolar CAS which suggests some small lack of homogeneity even at this composition, which may initiate phase separation within the CASs and could promote recrystallisation. Spectroscopic data help to identify the interaction between the two components in the form of two heterosynthons across the compositional range (10). The computational data provides the possibility of an additional heterosynthon, existing alongside the heterodimers in an equimolar system, where a VAL molecule can bond with two molecules of NIC in a NIC-VAL-NIC three-molecule heterosynthon and the calculated IR data of three-molecular heterosynthon is a closer match to the experimental FT-IR data of the equimolar CAS, than that of the two heterodimers. Such a prevalence of the three-molecule heterosynthon could potentially leave some VAL unbonded within the equimolar co-amorphous system, making it inhomogeneous, possibly lead to phase separation and eventually recrystallisation over time and thus affecting its long-term stability.

Solubility studies indicate that co-amorphisation strategy significantly enhances the aqueous solubility of VAL. Although the aqueous solubility of the binary systems across the compositional space showed no significant difference, the equimolar MA CAS demonstrated the highest VAL solubility compared to all the other CASs suggesting the presence of optimal binding between the two components at this ratio. Stability studies under 85% RH demonstrated that the equimolar system happened to be the most recrystallisation resistant compared to the other systems, maintaining its amorphous state for 4 weeks by pXRD, whereas systems of higher NIC content recrystallised in a week with only NIC recrystallising. Through PLM, it was observed that the equimolar CAS retained the least crystallinity after 1 week of ageing, whereas systems with excess of either of the components demonstrated a rise in crystallinity. This is potentially due to the fact that this molar ratio facilitates the most uniform intermolecular interactions in the whole molar range.

Although the equimolar system does show signs of inhomogeneity by PLM and tmDSC, and it performs better than other systems in terms of stability and solubility and possesses the least retained crystallinity across the wide compositional space, thus making it the appropriate composition, thereby fulfilling the aims of this chapter.

## Chapter 5. Influence of preparation methods of co-amorphous systems on amorphisation, solubility and dissolution

### 5.1. Chapter Overview

The aim of this chapter is to study the effect of preparation technique on the structure, interaction, and performance of the equimolar co-amorphous systems of valsartan (VAL) and nicotinamide (NIC) and identify which method of preparation produces a system with the most enhanced solubility and dissolution profile compared to the semi-crystalline VAL. These involve thermodynamic approaches such as melt amorphisation, a thermal technique, slurry mixing, a solvent evaporation technique, spray drying, an atomising and solvent evaporation technique and ball milling, a mechanochemical process. Each of these CASs are also compared to the API in its amorphous form to study the effectiveness of the co-amorphisation strategy. The systems were analysed by pXRD, X-ray PDF, PLM, TGA and tmDSC and FT-IR and Raman spectroscopy, which indicated the differences in structure, retained crystallinity and interactions within the binary systems. Aqueous solubility and dissolution profiles of the systems were also analysed, which showed that the system with highest solubility was prepared by spray drying and the one with the best dissolution profile was prepared by melt amorphisation. BM CAS showed poor dissolution as well as the lowest solubility in comparison to all the four binary system. This chapter highlights the subtle differences in the four formulation techniques and how these differences could potentially affect their performances.

### 5.2. Introduction

From [Chapter 4](#), it was established that an equimolar system is the optimal molecular ratio for the preparation of stable VAL and NIC co-amorphous system. The system was prepared by the thermal technique of melt amorphisation. Here, VAL-NIC equimolar CASs are going to be prepared by different techniques. Different ways of preparing co-amorphous dispersions have been described (10, 12, 68, 260). The many preparation techniques can be broadly divided into two categories (11, 12, 261):

- Thermodynamic disarrangement: when a crystalline API is subjected to thermal methods such as melting or heating in a solution. In such a process, the melted system is rapidly cooled to retain the amorphous product (quench cooling), or the

API system is retained by solvent removal (vacuum evaporation) to obtain the amorphous API.

- Kinetic disarrangement: when a crystalline API system is subjected to mechanical forces to transform it into an amorphous state by introducing crystal defects and disorders, in processes such as ball milling.

As the research of co-amorphisation is in its early stages, there has not been enough information to fully learn about how different processing techniques affect the stability and the functionalisation of a particular co-amorphous system. Nor is it well-established how physically stable systems prepared by different techniques are against phase separation and recrystallisation. This chapter investigates the characteristics of four co-amorphous formulations prepared by different techniques based on kinetic and thermodynamic disarrangements, namely ball milled (BM), melt amorphised (MA), spray dried (SD) and slurry mixed (SL), as discussed in section 3.4 of Chapter 3. It will also investigate solubility and dissolution profiles in water in comparison to the semi-crystalline pure VAL by the methods presented in sections 3.5.5, 3.5.6, and 3.5.7. In addition, the semi-crystalline VAL was amorphised to study if the co-amorphous formulations perform better, worse or the same as the drug when prepared in the amorphous state.

### 5.3. Results and Discussion

In Chapter 4, it was established that equimolar system of VAL and NIC was the optimal ratio for having minimal retained crystallinity, having the highest VAL solubility and being the most recrystallisation resistant system across the molar range. Building on that, four single-phase equimolar co-amorphous systems of VAL and NIC were prepared by four different preparation techniques, namely melt amorphised (MA), ball milled (BM), slurry mixed (SL) and spray dried (SD) to study the increase in aqueous solubility and dissolution rate of the binary systems. The different processing techniques produced products that had subtle physical difference.

As mentioned in the last chapter, the MA CASs upon melting and cooling form a transparent mass on the glass vessel where it melted and quickly breaks into a fine white powder upon stress.

Ball milling was conducted for four hours, at one-hour intervals and tested by pXRD to see if the crystalline peaks of the two components can be detected. The

characteristic Bragg peaks of VAL decreased after an hour of milling whilst those of NIC were still present, but then gradually decreased with the increasing milling time. This suggests that VAL-NIC binary system underwent amorphisation upon milling. After 240 minutes of milling, all Bragg peaks have completely disappeared and the X-ray diffraction formed an amorphous halo with no peaks, indicating the formation of an amorphous system. Furthermore, a change in colour was observed, from white powdery mixture of VAL and NIC to a yellowish-white coloured powder left within the grinding jar. This could be due to mechanochemical activation of the mixture, transforming it from a crystalline mixture to an amorphous mixture and facilitating miscibility between the two components (262, 263). Another reason for the colour change could be due to degradation of the material, but further investigation does not suggest such a phenomenon.

The SL CAS formed a shiny white powder upon drying in the rotary evaporator. Under vacuum and at 60 °C, the solution thickened up like a sticky gel and started to foam inside the round bottom flask soon after, without boiling over. The system maintained a porous appearance even after the ethanol had completely evaporated. To extract the powder from the flask, a spatula was inserted and on doing so, the white porous network of powder crumbled to the bottom of the flask, where it was extracted and kept in a desiccator for further investigation.

The SD CAS formed a white powder on the walls of the cyclone of the instrument. Not enough sample accumulated in the sample collector of the spray dryer. This is probably because the SD CAS was very sticky, and it was difficult to remove from the cyclone, and it did not slide into the collection jar. The powder was scraped off and in doing so, charge was building up, leading to clustering of the powders at the end of the spatula and within the glass vessel. The mechanical stress applied to separate the powder may have affected the structure.

All of these powders were stored in a desiccator before they were characterised and analysed.

### 5.3.1. Powder X-Ray Diffraction (pXRD)

The pXRD patterns of equimolar CASs of VAL and NIC prepared by BM, MA, SD, and SL are presented in [Figure 5.1](#). pXRD data of the NIC can be accurately matched to the simulated pXRD pattern generated from the CCDC Database (CSD – refcode NICOAM – deposition number 1219542) using the CrystalDiffract software (within the

CrystalMaker software suite) (246). pXRD data of the semi-crystalline (SC) VAL, suggests a partially crystalline material that can be accurately matched with the pXRD pattern reported by (244, 245). This is evidenced by the presence of two sharp peaks at  $5.6^\circ$  and  $17.7^\circ$  and its partial amorphisity is indicated by the diffused peaks at  $15^\circ$  to  $25^\circ$ . The pXRD patterns of CASs and amorphous VAL (Am VAL) are different from the physical mixture (PM) of VAL and NIC as well as the as-bought components. The physical mixture shows peaks that reflects both VAL and NIC, with peaks at  $5.6^\circ$  and  $17.6^\circ$  from VAL and peaks at  $14.8^\circ$ ,  $13.5^\circ$ ,  $22.2^\circ$ ,  $23.3^\circ$ ,  $25.8^\circ$  and  $27.3^\circ$  from NIC. In the amorphous CASs, characteristic Bragg reflections of both the components are no longer present. Instead, an amorphous halo with two small humps is present in the CASs' X-ray patterns. The X-ray pattern of Am VAL exhibits the same appearance as the CASs. Such an appearance of an amorphous halo in the pXRD patterns in the binary CASs and the amorphous VAL demonstrate a lack of crystallinity within the systems.

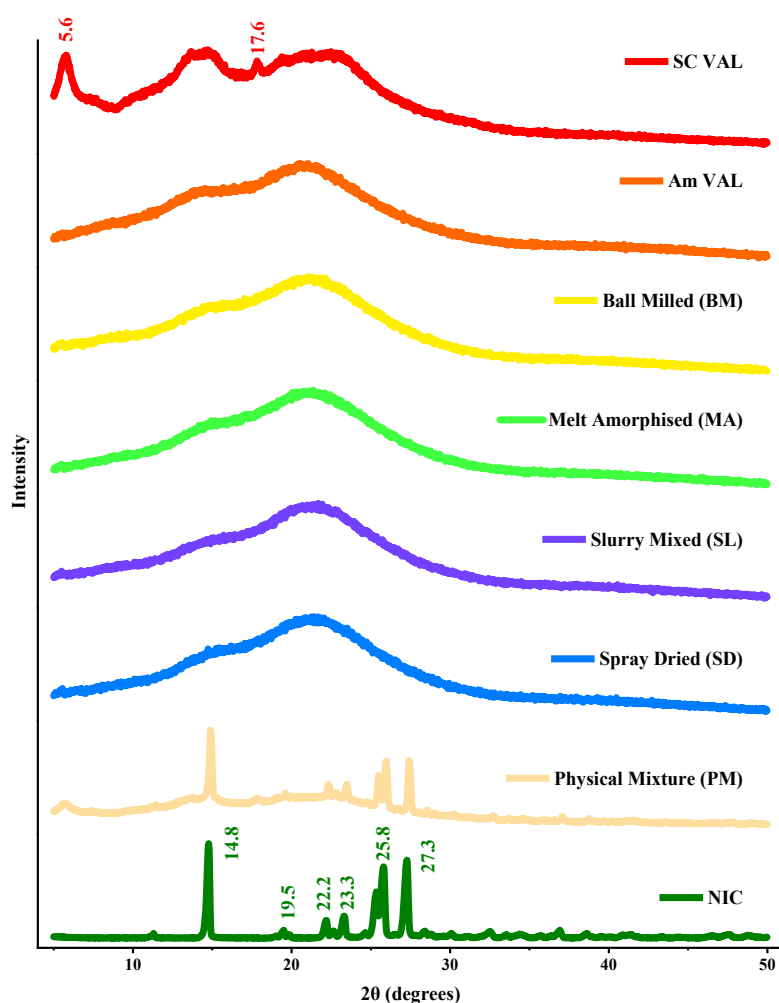


**Figure 5.1:** PXRd patterns of SC VAL, Am VAL, NIC and VAL-NIC CASs prepared by ball milling, melt amorphisation, slurry mixing and spray drying.

From the X-ray patterns, it can be inferred that all the four CASs are amorphous and the mechanical stress that was applied to them during extraction has not caused detectable recrystallisation.

### 5.3.2. Thermal Analysis

The thermal behaviour of the co-amorphous formulations was investigated by TGA and tmDSC and compared to that of the individual components and their physical mixtures.

#### 5.3.2.1. Thermogravimetric Analysis (TGA)

TGA has been done to observe any changes in mass with increasing temperature and first derivatives generated to identify transition points which may not be observable in the as acquired thermograms.

The TGA thermogram for SC VAL in Figure 5.2 (a), demonstrates three mass loss regions. The first one occurs between 45 to 90 °C, with a small mass loss of 0.2%, followed by the mass loss of 13.4% between 155 to 270 °C, potentially due to the decarboxylation of VAL, followed by the third and the largest mass loss of 75.7% between 270 to 450 °C. The first mass loss can be attributed to the loss of moisture absorbed by the VAL while the other two mass losses occur due to the thermal decomposition of the VAL (247, 248, 264). The TGA thermogram of NIC in Figure 5.2 (b), shows one mass loss event of 99.6% which occurs between 155 to 250 °C and can be attributed to its thermal decomposition at that temperature.

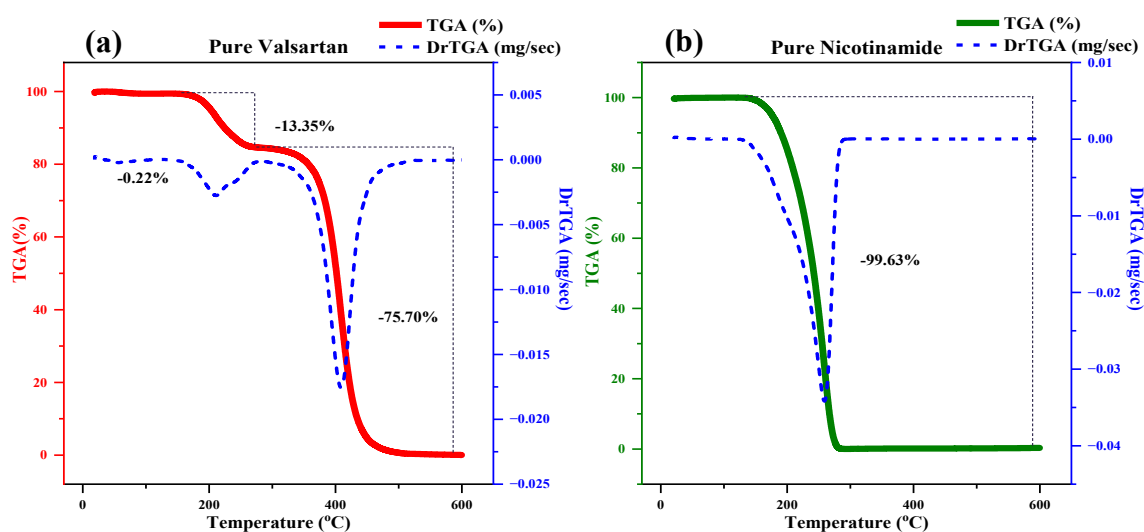


Figure 5.2: (a) and (b) TGA thermograms of pure valsartan and pure nicotinamide.

Figure 5.3 demonstrates the TGA thermograms of the four CASs. The losses observed in all the systems appears to follow the same pattern as that of VAL in Figure 5.2 (a). The TGA thermogram of CASs has three mass loss regions at 45 to 90 °C, 150 to 270 °C and 270 to 450 °C. However, on closer inspection, systems prepared by the solvent evaporation techniques, namely SD and SL have an additional mass loss region between 20 to 55 °C, that can be attributed to release of ethanol. This mass loss is approximately 0.15% and 0.40% for SD and SL, respectively. Such a mass loss cannot be assigned to MA and BM CASs. The first mass loss for BM and MA CASs can be attributed to moisture loss, at 0.05% and 0.35% respectively between 45 to 100 °C. Additionally, the moisture induced mass loss for SD and SL CASs are small as well, at 0.01% and 0.05%, respectively.

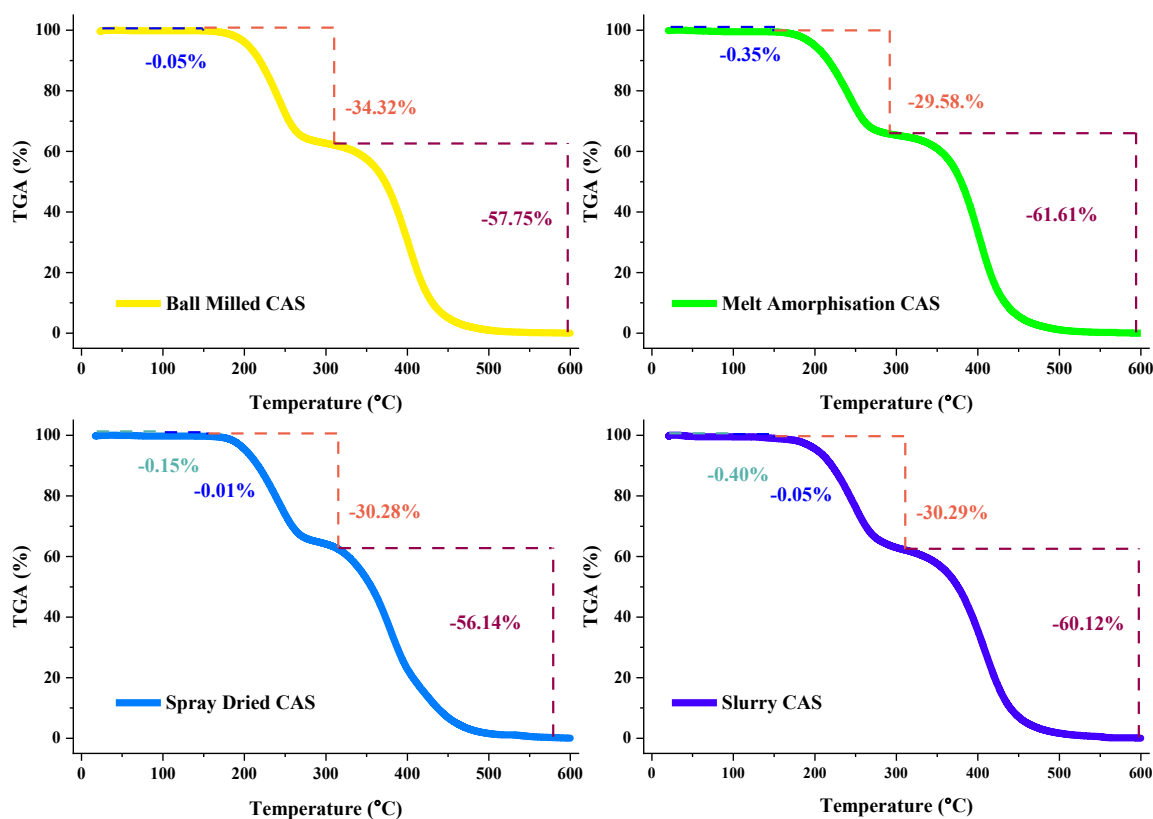


**Figure 5.3:** TGA of co-amorphised formulations of VAL and NIC. Percentages in blue and green represent moisture loss and release of ethanol, respectively. Percentages in orange and maroon represent mass loss due to thermal decomposition.

The next two large mass loss regions following the mass losses due to solvent release are similar across all CASs, all of them commencing at 155 °C. BM CAS shows the highest mass loss in this region, at 34.3% and MA CAS demonstrates the least, at 29.6%, in comparison to all for CASs. SD and SL CASs, show the same loss of 30.3%.

The third mass loss region commencing at 303 °C, for MA and SL CASs is approximately 61%, whilst BM and SD CASs are around 57%. This mass loss is attributed to the last thermal degradation for VAL and is reflected through all the CASs. The mass loss trend due to thermal degradation differs slightly from each other due to small differences in the CASs prepared by different methods, where the two components in the interact with each other differently in each CAS, possibly due to unique chemical reaction between the drug and the excipient in each of the CASs that can be detected by this thermal analysis, which may impact the stability and the performance of the binary systems (265).

Thus, TGA supports two findings. First, a small amount of weight loss is due to ethanol is present in all the CASs prepared with retained ethanol, namely the SD and SL CASs. Second, the small differences in the mass losses in the CASs prepared by different techniques can be due to different intensity of interaction between the components, which potentially leads to different chemical reaction between the components that which can impact its performance and stability.

#### 5.3.2.2. Temperature Modulated Differential Scanning Calorimetry (tmDSC)

tmDSC divides the total heat-flow rate, the one usually observed in normal DSC, into two heat-flows, namely reversing and non-reversing heat-flows to better separate kinetic events like crystallisation or enthalpy relaxation effects from reversible, heat capacity changes.  $T_g$  is determined by analysing the reversing heat-flow (250).

In [Figure 5.4](#), the anhydrous glass transition temperatures ( $T_g$ ) of the SC VAL, Am VAL, and the four co-amorphous formulations obtained from the second heating cycle have been presented. The first heating cycle that tracked events such as the hydration of the main CAS and minor phase separation of components (with or without recrystallisation) are reported in [Appendix B](#).  $T_g$ s from the first heating cycle are lower than the second heating cycles for all CASs, due to removal of trapped moisture and some further mixing, as discussed in [Chapter 4](#). All of the CASs demonstrate a singular  $T_g$  other than the MA CAS, in the first heating cycle. The MA and BM CASs also had melting endotherms of NIC and VAL respectively, indicating the presence of small levels of crystallinity whereas the solvent evaporated CASs showed no melting endotherm in both the heating cycles, suggesting the total lack of any crystallinity.

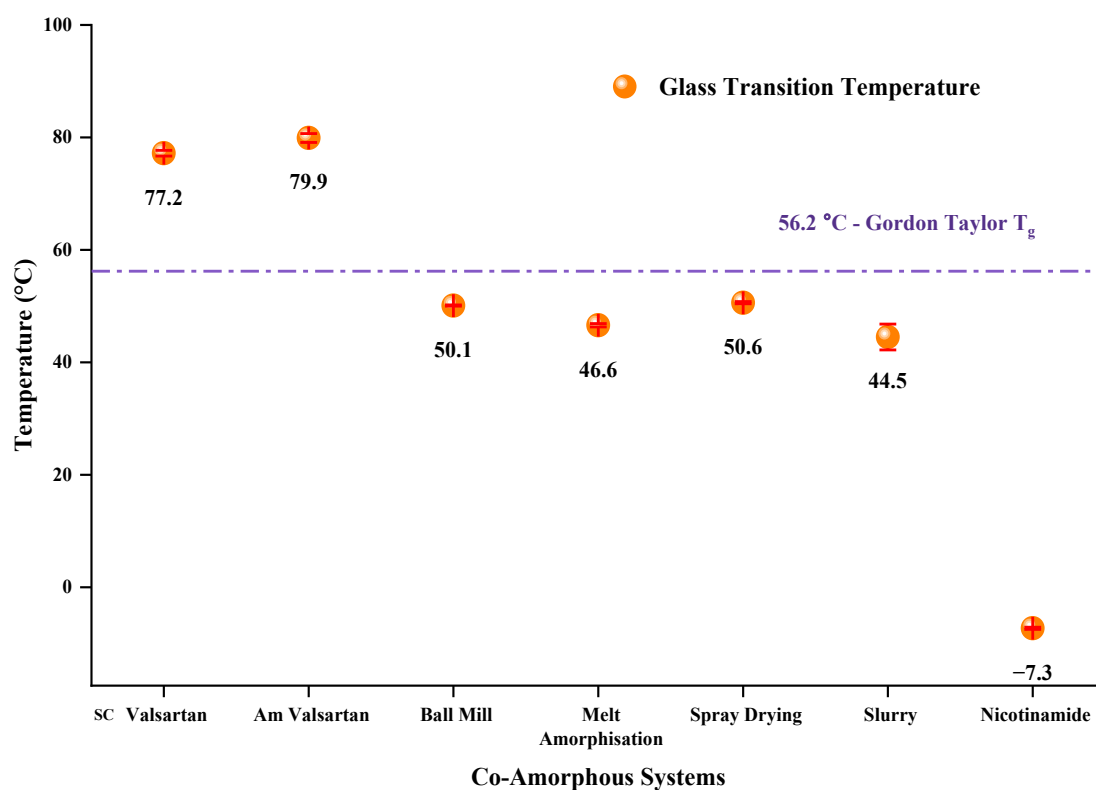


**Figure 5.4:** Anhydrous glass transition temperatures ( $T_g$ s) of SC VAL, Am VAL, NIC, and CASs prepared by BM, MA, SD and SL, obtained from the second heating cycle. The expected  $T_g$  for an equimolar system by the GT equation is presented as a purple line at 56.2 °C. Each experimental data point is an average of three repeats along with a standard deviation depicted by the error bars.

The Gordon Taylor (GT) equation has been implemented to determine  $T_g$ s of the binary co-amorphous systems. The  $T_g$  of VAL has been determined to be  $77.2 \pm 0.5$  °C by means of tmDSC as well as standard DSC, as shown in [Chapter 4](#). Zhou *et al* had determined its  $T_g$  to be  $-7.3 \pm 0.2$  °C, by preparing a fully amorphous NIC by quenching the excipient in liquid nitrogen (149, 251). Based on the experimental  $T_g$  values of VAL and theoretical  $T_g$  value of NIC, the calculated  $T_g$  for 1:1 CAS is 56.2 °C. This was then compared to the experimental  $T_g$  values of the produced anhydrous CASs.

It is crucial to understand the relationship between the  $T_g$  of a binary system and the molar ratio, as it is may sometimes be difficult to produce a homogeneous amorphous system. The SC VAL had two endothermic events, namely the glass transition event and melting event, whereas the Am VAL prepared by melt amorphisation has a singular endothermic glass transition event, with a  $T_g$  value of  $79.9 \pm 0.8$  °C, slightly higher than that of the SC VAL. The four CASs have only one endothermic event, i.e., the glass transition event, as expected due to their amorphous nature. The absence of melting

endotherms for both components in the binary systems, along with the presence of a single  $T_g$  indicates the presence of intermolecular interaction between VAL and NIC as well as the lack of crystallinity. The SD CAS has the highest  $T_g$  value at  $50.6 \pm 0.2$  °C, followed by BM, MA, and SL CASs at  $50.1 \pm 0.1$ ,  $46.6 \pm 0.3$  and  $44.5 \pm 2.3$  °C respectively.

This higher value of  $T_g$  for the BM and SD CASs, being closer to the calculated value could potentially be due better mixing which shifts the value closer to the calculated  $T_g$ . Further analysis would assist in understanding this discrepancy more.

### 5.3.3. Microscopy

#### 5.5.3.1. Polarised Light Microscopy (PLM)

Polarised light microscopy (PLM) gives us the insight into crystallinity within the systems, namely that of the pure components and the co-amorphous systems. Crystallinity was observed here in the form of birefringence within the solid amorphous particles.

Figure 5.5 shows the particles of VAL and NIC co-amorphous systems prepared by different techniques at 10x magnification, starting from SC VAL, Am VAL, BM CAS, MA CAS, SD CAS and SL CAS. Figure 5.5 starts with an image of SC (as-bought) valsartan powder, placed over a glass slide. Birefringence is observed in some isolated, irregularly faceted particles, confirming crystallinity in as-bought VAL. The Am VAL does not demonstrate such birefringence indicating the lack of crystallinity in the system.

Below the images of SC VAL and Am VAL, are the images of the four co-amorphous formulations in Figure 5.5. For the BM CAS, NIC crystals appear to exist outside of the amorphous particles, as indicated by the presence of sharp needles existing on their own, without being bonded to any particles, which suggests inhomogeneity in the system. Such an occurrence was observed in multiple images of this system. This lack of homogeneity can be attributed to the kinetic disarrangement mode of preparation, which led to clustering of individual components during the synthesis, facilitated by the charge build during the clashing of the steel balls in the grinding jar with each other as well as the walls of the jar. Although the system is X-ray amorphous, separate NIC crystallinity is clearly present throughout this system. The lack of homogeneity in the BM CAS suggests that the two components can exist in the amorphous form as well as have low levels of crystallinity, which may affect their structure as well as their glass transition values, as seen in section 5.3.2.2.

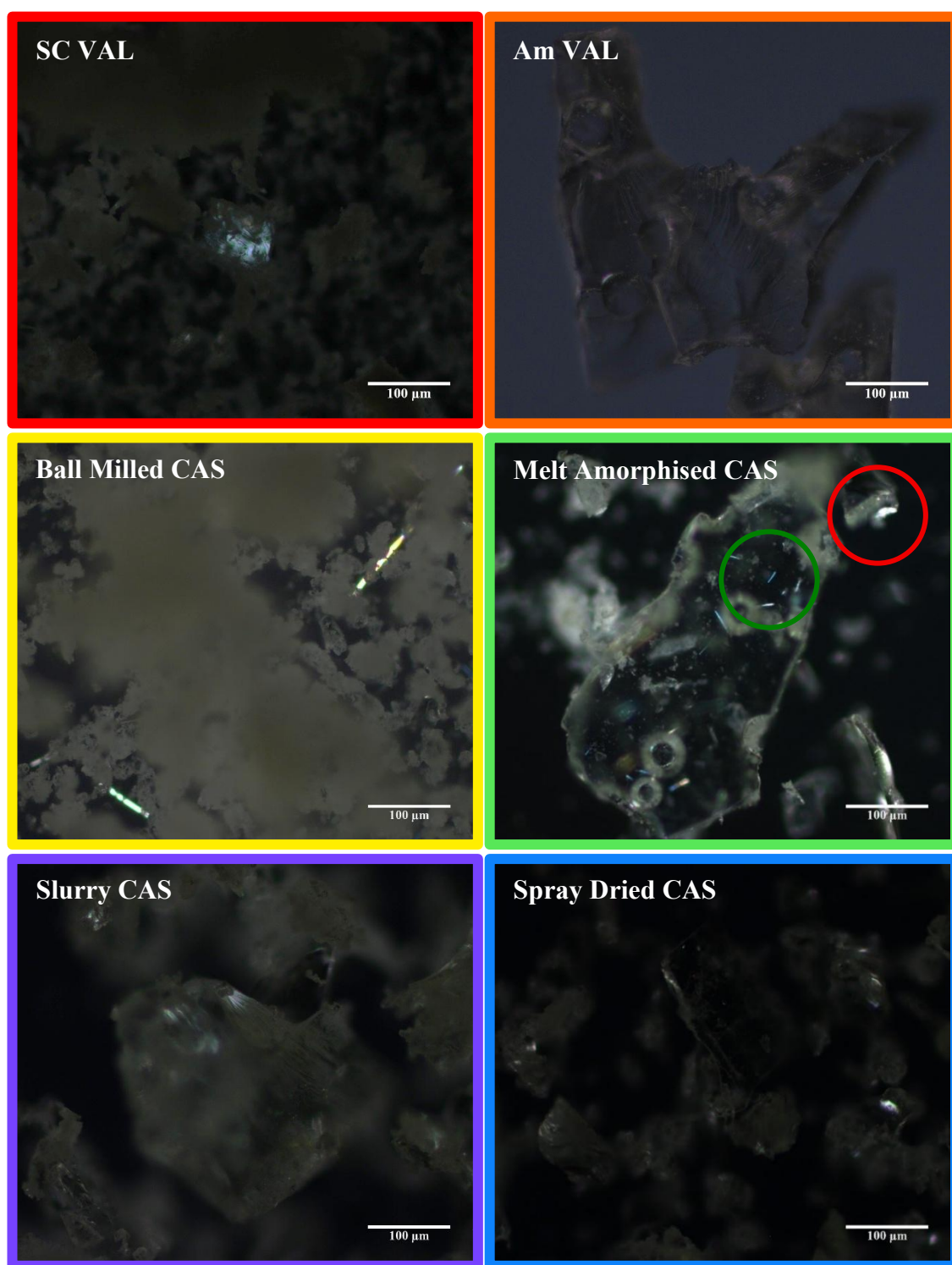


**Figure 5.5:** PLM images of SC VAL, Am VAL and VAL-NIC CASs, namely BM, MA, SL and SD CASs. The birefringence circled in red and green signify VAL and NIC crystallinity, respectively.

The next image is that of an equimolar MA CAS. In this system, NIC crystallinity (in green circle) is present within the particles in the form of small needles, evidenced by multiple images. Some VAL crystallinity (in red circle) can also be observed in these particles. However, the systems do not appear to be as phase separated like the BM CAS, suggesting lower levels of inhomogeneity in this otherwise X-ray amorphous binary

system. This could be due the fact that this was prepared kinetically and thermodynamically, with the components ground together and then heated at a temperature higher than the melting point of both the components, which facilitates further mixing of systems at a molecular level.

The next two images are that of the SL and SD CASs. Both these systems have been prepared by a solvent evaporation technique, a thermodynamic disarrangement method. For the SL CAS, birefringence can hardly be observed in the system, and is there in very low amounts, making it harder to assign the birefringence to any one component. Not only is this an X-ray amorphous CAS, it also is amorphous to the PLM technique. The SD CAS is similar to the SL CAS, with very low amounts of birefringence, but slightly higher than that of the SL CAS, it is either difficult to assign the crystallinity to either of the components or the crystallinity can be assigned to both the components, with no one component being more dominant than the other, indicating homogeneity is present throughout the system. However, this slight difference to otherwise X-ray amorphous solvent-based CASs can be attributed to the difference in the solvent used during the preparation. The SL CAS was made with absolute ethanol whereas a solvent mixture (50/50 v/v) of water and 98% ethanol was used in the spray dried technique. The presence of such a high volume of water suggests that some water molecules may have gotten trapped in the amorphous particles, which may have led to small-scale recrystallisation within the SD CAS.

To understand the subtle differences in retained crystallinity in the four differently prepared CASs, the retained crystallinity of the systems was quantified by evaluating the percentage of crystallinity in the samples with respect to area. 10 images of each of the samples were used to study their total crystallinity and has been presented in [Figure 5.6](#). From the [Figure 5.6](#), it is very clear that slurry mixed CAS has no crystallinity whereas the other three CASs have low levels of crystallinity. The SD CAS has the lowest levels of crystallinity at 0.2%, right after SL CAS, followed by MA and BM CASs at 1.11% and 1.55% crystallinity, respectively. Upon quantifying the crystallinity, it has become clear that the CASs produced in the absence of solvents have higher crystallinity due to phase separation than the solvent-based CASs. This is possibly due to the formation of homogeneous solutions in the respective solvent/solvent mixture prior to removal of solvents under heat and pressure to obtain the CASs, which develops better interaction between the two components, thus leading to higher stability against phase separation and

recrystallisation in comparison to the systems that are produced by mechanochemical processing or just thermal processing.

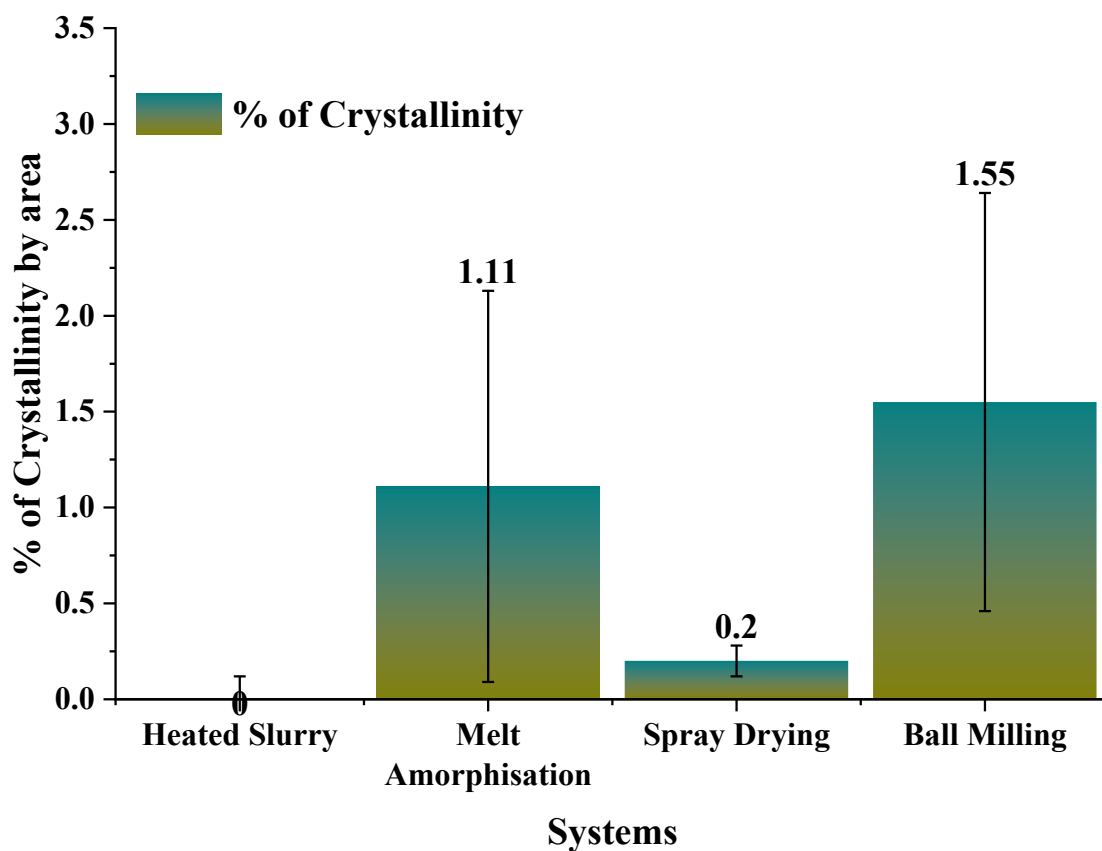


**Figure 5.6:** Percentage of crystallinity by area in the co-amorphous formulations. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size, with the error bars depicting the standard deviation.

PLM detects some retained crystallinity in the otherwise X-ray amorphous systems. Phase separation is more dominant in thermal and kinetic (MA and BM) CASs which cannot be observed in solvent-based (SL and SD) CASs, further supporting the findings of tmDSC in section 5.3.2.2. Upon closer inspection of the two solvent-based CASs, the system prepared with no water (SL) has little to no crystallinity than the system prepared with water in the solvent mixture (SD), where the latter demonstrates some retained crystallinity.

### 5.5.3.2. Scanning Electron Microscopy (SEM)

The powder morphology of the systems and the starting components aid in the preliminary evaluation of the type of solid-state form present in each material.

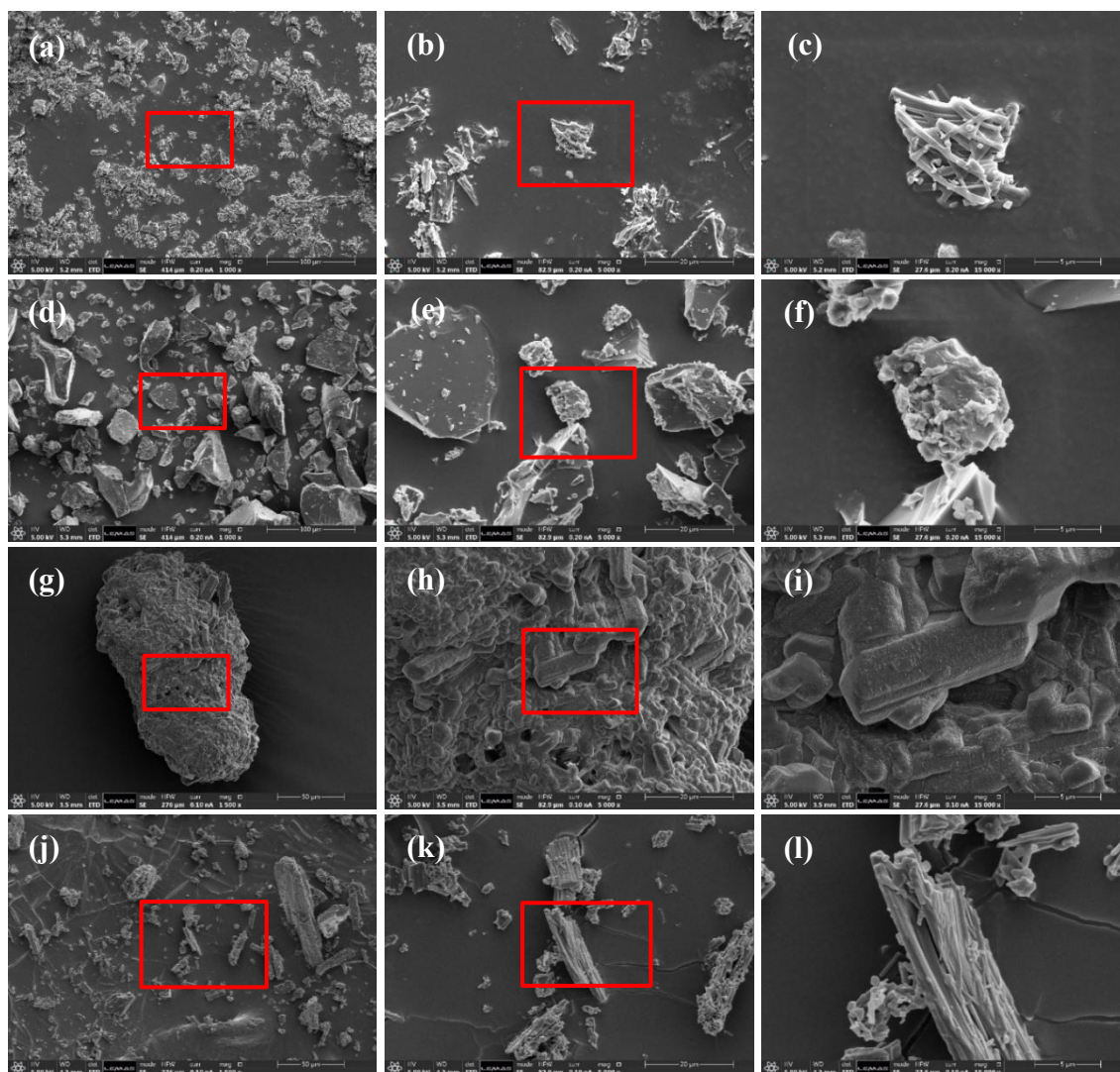


**Figure 5.7:** (a, b, c) SEM images of SC valsartan at 1000, 5000 and 15000 magnifications. (d, e, f) SEM images of prepared Am valsartan at 1000, 5000 and 15000 magnifications. (g, h, i) SEM images of crystalline nicotinamide at 1500, 5000 and 15000 magnifications. (j, k, l) SEM images of the physical mixture of valsartan and nicotinamide at 1500, 5000 and 15000 magnifications.

The SEM images of SC VAL, Am VAL, NIC, and their physical mixture are presented in Figure 5.7. The semi-crystalline VAL imaged at varying magnifications demonstrated in Figure 5.7 (a, b, c) has particles that are irregularly shaped, often resembling a long rectangular structure, with the particles aggregating, giving a rough external appearance. They have a varying size, from 3  $\mu\text{m}$  to 37  $\mu\text{m}$ . The Am VAL images in Figure 5.7 (d, e, f) are slightly different from the semi-crystalline VAL, the surface roughness is far less compared to its predecessor. The particles are irregularly shaped,

with sizes varying from 1  $\mu\text{m}$  to 65  $\mu\text{m}$ . [Figure 5.7 \(g, h, i\)](#) shows images of NIC in the form of spherical aggregates with high surface roughness in the form of small rods. The physical mixture of VAL and NIC in [Figure 5.7 \(j, k, l\)](#) show aggregated particles of varying sizes from 5  $\mu\text{m}$  to 60  $\mu\text{m}$ , with irregular, rectangular shapes, and high surface roughness.

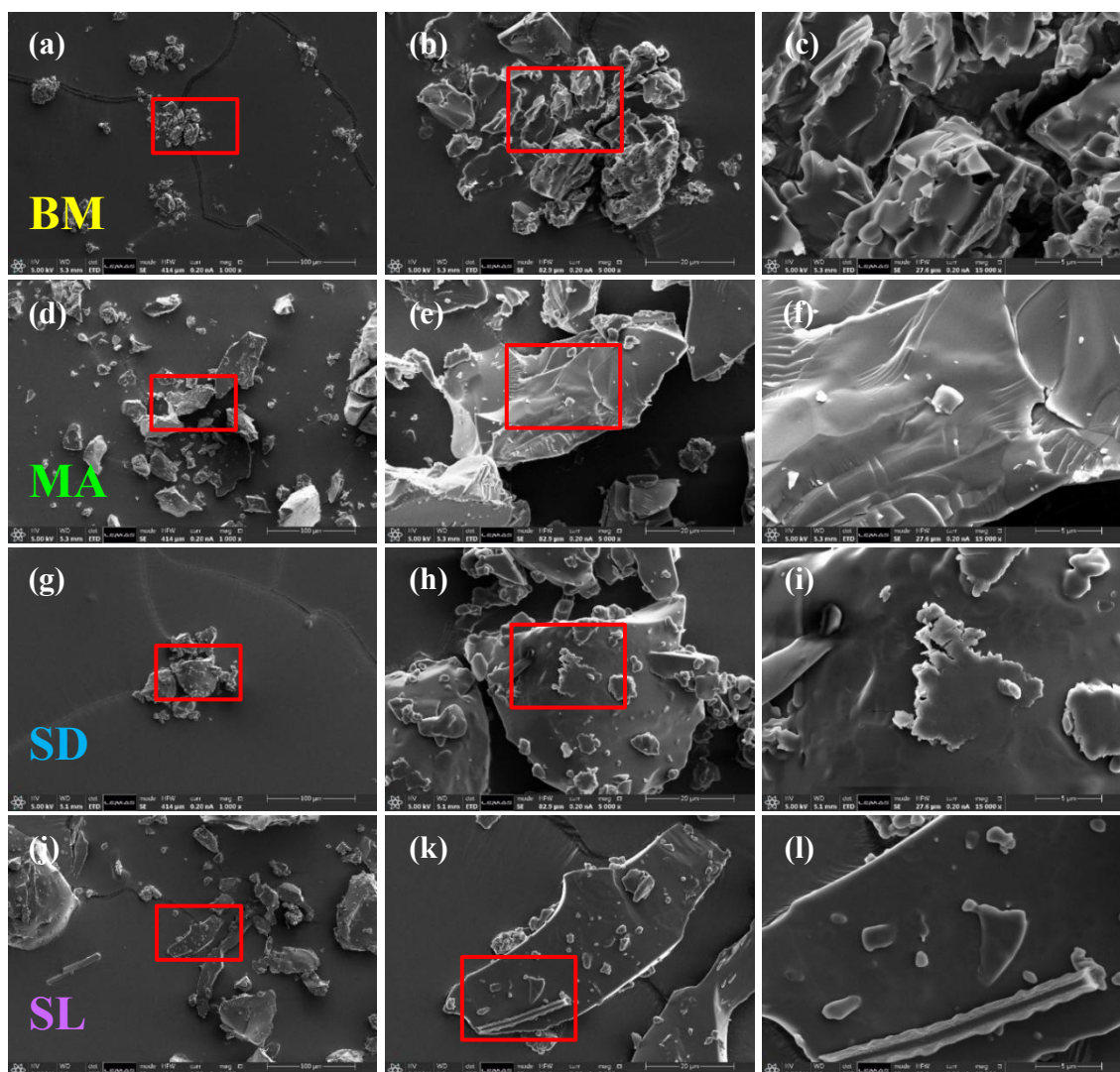


**Figure 5.8:** (a, b, c) SEM images of BM CAS at 1000, 5000 and 15000 magnifications. (d, e, f) SEM images of MA CAS at 1000, 5000 and 15000 magnifications. (g, h, i) SEM images of SD CAS at 1000, 5000 and 15000 magnifications. (j, k, l) SEM images of SL CAS at 1000, 5000 and 15000 magnifications.

The CASs have distinct morphology differences from the pure components and their PM as demonstrated in [Figure 5.8 \(a to l\)](#), at magnifications 1000, 5000 and 15000. All the four formulations have a glass-like appearance in comparison to the drug or its physical mixture with NIC. The particles appear to be of homogeneous structure, with the particle sizes varying from 1  $\mu\text{m}$  to 70  $\mu\text{m}$ , across all the formulations. There appears to

be no distinct morphological differences between the CASs prepared by different methods.

SEM images show the distinct difference in the surface of the particles of the as-bought components and their co-amorphous mixture, with the former being aggregates, having higher surface roughness and the latter being homogeneous, having a lower surface roughness.

### 5.3.4. Infrared Spectroscopy

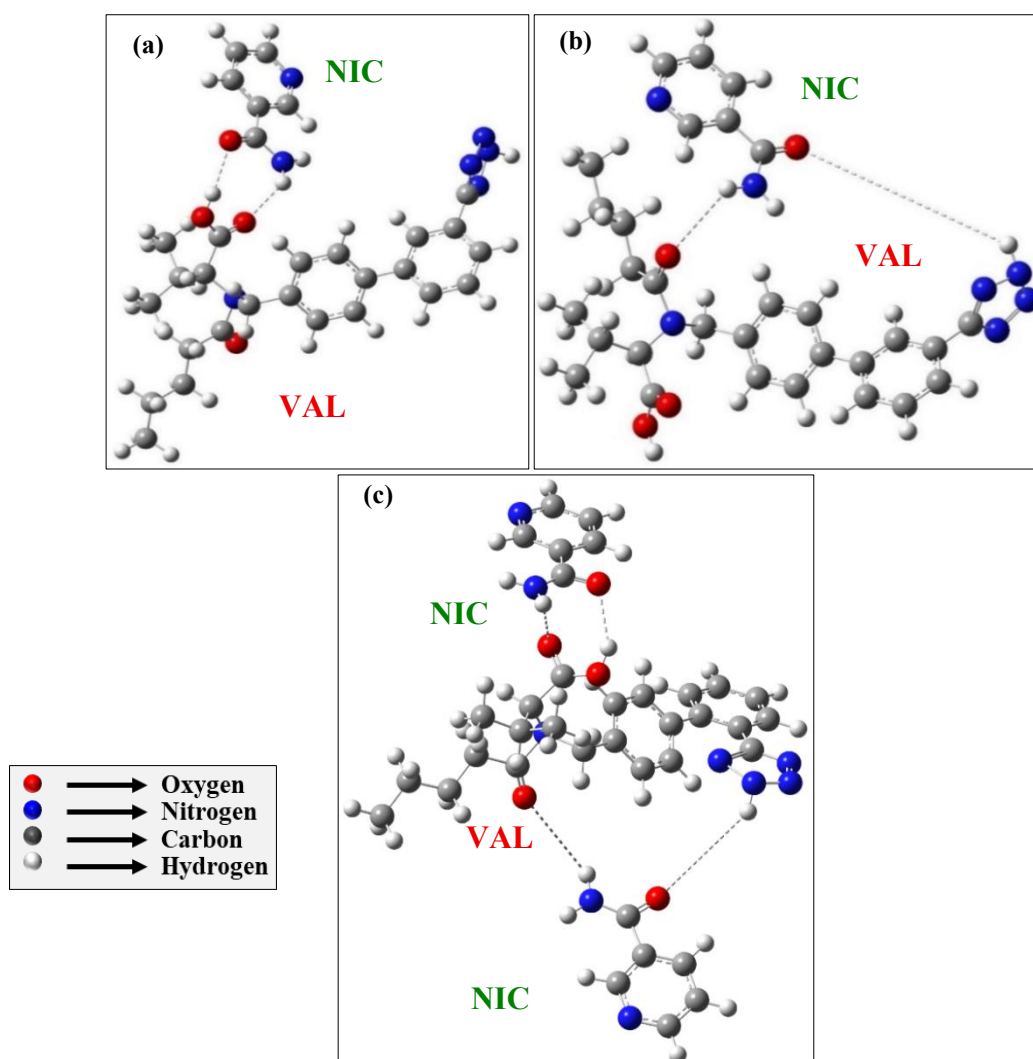


**Figure 5.9:** (a) VAL-NIC1 (acid-amide – N-HNIC-amide---OVAL<sup>-</sup> and O-H---ONIC-amide) heterosynthon. (b) VAL-NIC2 (tetrazolyl-amide-amide – N-HVAL-tetrazolyl---ONIC-amide and N-HNIC-amide---OVAL-amide) heterosynthon. (c) NIC-VAL-NIC system is a combination of the two idealised conformation VAL-NIC1 and VAL-NIC 2. Modelled and optimised in GaussView 6.1.1.

The infrared spectroscopy analysis of the co-amorphous formulations and their starting components are presented in this section. DFT-based computational figures of VAL-NIC heterosynthons from Chapter 4 are presented in Figure 5.9. FT-IR is able to

detect the bonding present in VAL-NIC1, as shown in Figure 5.9 (a) and Raman detects the *additional* bonding interaction of VAL-NIC2, presented in Figure 5.9 (b), although this heterosynthon is may not energetically viable, as discussed in section 4.3.4. Figure 5.9 (c) demonstrates the third kind of bonding interaction, where two NIC molecules might bind with one molecule of VAL, with the bonding sites of the two heterodimers.

#### 5.3.4.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy has been employed on the pure components and the four CASs to study the component interaction within the CASs by comparing to spectra of the pure components.

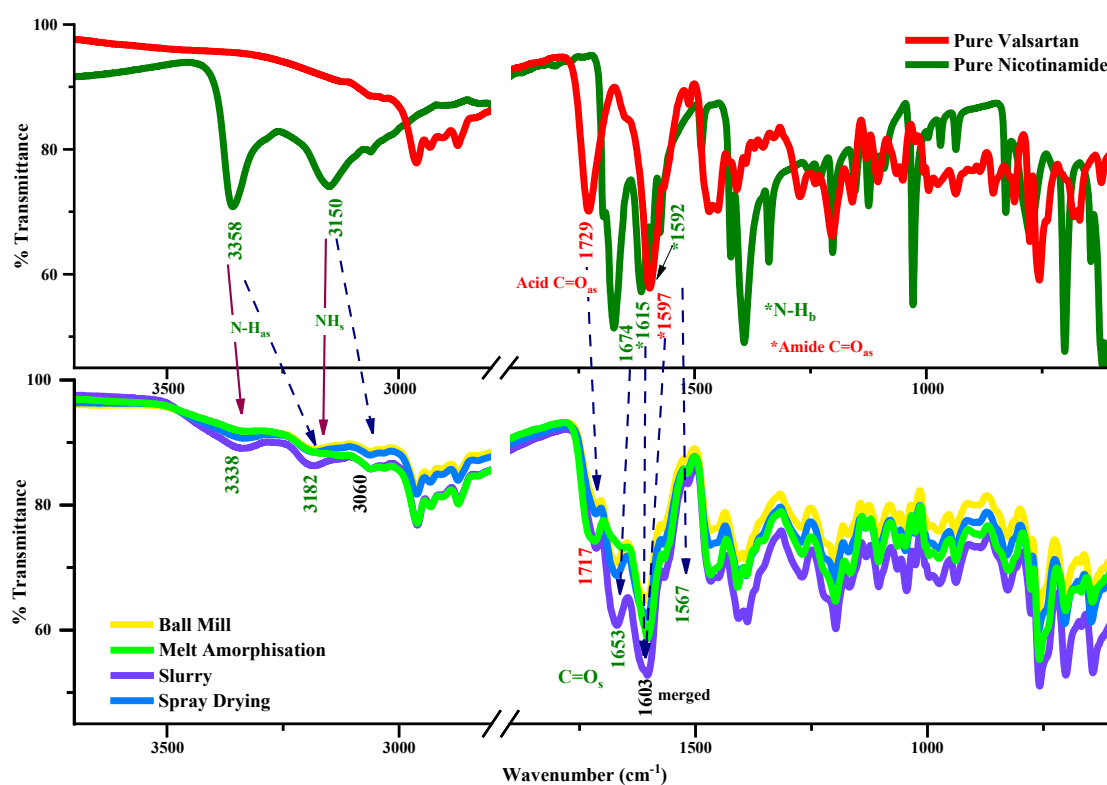


**Figure 5.10:** FT-IR spectra for as-bought VAL and NIC are overlapping each other, stacked over the overlapped spectra of the four co-amorphous formulations.

The FT-IR spectra of VAL, NIC and the co-amorphous formulations are presented in **Error! Reference source not found.**. The peaks in the amorphous CAS FT-IR spectra are broader than that of the starting components, a common occurrence in amorphous form products (142, 256). From Chapter 4, we established that four types of hydrogen bonds can exist in the CAS between VAL and NIC. They can form between the amide group of NIC and acid group of VAL, as in the case of VAL-NIC1 heterodimer, demonstrated in Figure 5.9 (a). The other two hydrogen bonds can form between the

amide group of NIC with the tetrazolyl H of VAL and carbonyl group of VAL, as demonstrated in Figure 5.9 (b). The FT-IR spectrum of VAL demonstrates two typical sharp peaks in  $1729\text{ cm}^{-1}$  and  $1597\text{ cm}^{-1}$ , relating to the carbonyl (C=O) in acid and (C=O) in amide stretching vibrations, respectively. The broad peak with maxima at  $3120\text{ cm}^{-1}$  has been assigned to the OH stretching vibration of VAL. A weak absorption band at  $3455\text{ cm}^{-1}$  for NH tetrazole has been detected in the VAL spectrum. The FT-IR spectrum of NIC shows two signature peaks at  $3358\text{ cm}^{-1}$  and  $3150\text{ cm}^{-1}$ , which can be assigned to the asymmetric and symmetric stretching vibrations of  $\text{NH}_2$  groups, at  $1674\text{ cm}^{-1}$  (with a shoulder at  $1696\text{ cm}^{-1}$ ) corresponds to the stretching vibration of carbonyl C=O and peaks at  $1615\text{ cm}^{-1}$  and  $1575\text{ cm}^{-1}$  correspond to the bending vibration of NH. The changes in wavenumbers of peaks in the functional group which forms hydrogen bonds between the components the four equimolar co-amorphous formulations are highlighted in blue in Error! Reference source not found.. A comparative analysis of the absorption bands of the H-bond forming functional groups of the starting compounds with that of the CASs demonstrates clear trends in the peak shifts, suggesting the detection of intermolecular hydrogen bonds between the two starting compounds (10).

**Table 5.1:** Experimental Values of FT-IR spectra for VAL, NIC, and co-amorphous systems along with shifts ( $\Delta$ ) relative to VAL and NIC. The functional groups highlighted in blue are potentially involved in hydrogen bonding. The VAL and NIC values are in red and blue respectively, mirroring the data in Error! Reference source not found..

| <b><u>Functional Groups</u></b>     | <b><u>Frequency (<math>\text{cm}^{-1}</math>)</u></b> |                   |                          |                          |                          |                          |
|-------------------------------------|-------------------------------------------------------|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                                     | <b><u>VAL</u></b>                                     | <b><u>NIC</u></b> | <b><u>BM CAS</u></b>     | <b><u>MA CAS</u></b>     | <b><u>SD CAS</u></b>     | <b><u>SL CAS</u></b>     |
| (O-H) stretching                    | 3116                                                  | -                 | 3183<br>( $\Delta=+57$ ) | 3174<br>( $\Delta=+59$ ) | 3182<br>( $\Delta=+66$ ) | 3181<br>( $\Delta=+55$ ) |
| $\nu(\text{NH})_{\text{tetrazole}}$ | 3455<br>(poor signal)                                 | -                 | -                        | -                        |                          |                          |
| Acid (C=O) stretching               | 1729                                                  | -                 | 1713<br>( $\Delta=-16$ ) | 1717 ( $\Delta=-12$ )    | 1713 ( $\Delta=-16$ )    | 1713<br>( $\Delta=-16$ ) |
| Amide (C=O) stretching              | 1597                                                  | -                 | 1603<br>(Shoulder        | 1603<br>(Shoulder        | 1603<br>(Shoulder        | 1603<br>(Shoulder        |

|                                      |      |                               |                                           |                                    |                                    |                                           |
|--------------------------------------|------|-------------------------------|-------------------------------------------|------------------------------------|------------------------------------|-------------------------------------------|
|                                      |      |                               | at 1616)<br>( $\Delta=6$ )                | at 1612)<br>( $\Delta=6$ )         | at 1625)<br>( $\Delta=6$ )         | at 1616)<br>( $\Delta=6$ )                |
| $\nu(\text{C-O})$                    | 1390 | -                             | 1389<br>( $\Delta=-1$ )                   | 1389 ( $\Delta=-1$ )               | 1388 ( $\Delta=-2$ )               | 1388<br>( $\Delta=-2$ )                   |
| Unreacted<br>(N-H) as.<br>stretching | -    | 3358                          | 3336<br>( $\Delta=-22$ )                  | 3358                               | 3336 ( $\Delta=-22$ )              | 3358                                      |
| (N-H) as.<br>stretching              | -    | 3358                          | 3183<br>( $\Delta=-175$ )                 | 3174 ( $\Delta=-184$ )             | 3182 ( $\Delta=-176$ )             | 3181( $\Delta=-177$ )                     |
| (N-H)<br>stretching                  | -    | 3150                          | 3061<br>( $\Delta=-88$ )                  | 3060 ( $\Delta=-90$ )              | 3062 ( $\Delta=-88$ )              | 3062<br>( $\Delta=-88$ )                  |
| (C=O)<br>stretching                  | -    | 1674<br>(shoulder<br>at 1696) | 1668<br>( $\Delta=-6$ )                   | 1653 ( $\Delta=-21$ )              | 1668 ( $\Delta=-6$ )               | 1665<br>( $\Delta=-9$ )                   |
| (N-H) bending                        | -    | 1615,<br>1575                 | 1603,<br>1567<br>( $\Delta=-12$ , -<br>8) | 1603,<br>1567 ( $\Delta=-12$ , -8) | 1603,<br>1567 ( $\Delta=-12$ , -8) | 1603,<br>1567<br>( $\Delta=-12$ , -<br>8) |
| $\nu(\text{C-N})$                    | -    | 1340                          | 1271<br>( $\Delta=-69$ )                  | 1269 ( $\Delta=-71$ )              | 1270 ( $\Delta=-70$ )              | 1270<br>( $\Delta=-70$ )                  |
| $\rho_{\text{w}}(\text{NH}_2)$       | -    | 777<br>( $\Delta=0$ )         | 777<br>( $\Delta=0$ )                     | 777 ( $\Delta=0$ )                 | 777 ( $\Delta=0$ )                 | 777<br>( $\Delta=0$ )                     |

The OH stretching peaks of VAL of the BM, MA, SD and SL CASs have shifted to a higher value of 3183  $\text{cm}^{-1}$ , 3174  $\text{cm}^{-1}$ , 3182  $\text{cm}^{-1}$  and 3181  $\text{cm}^{-1}$  respectively from 3116  $\text{cm}^{-1}$ , which is potentially due to the decrease in bond length in the OH bond, as the H potentially is bonded to the amide O of NIC forming a O-H-O synthon, while the C=O peaks of the CASs (in the same order) of NIC have shifted to lower values, from 1674  $\text{cm}^{-1}$  to 1668  $\text{cm}^{-1}$ , 1653  $\text{cm}^{-1}$ , 1668  $\text{cm}^{-1}$  and 1665  $\text{cm}^{-1}$  respectively, probably due to a bond lengthening occurring with this hydrogen bond. Furthermore, both the NH stretching vibrations have shifted to lower values, from 3358  $\text{cm}^{-1}$  and 3150  $\text{cm}^{-1}$  to

around  $3182\text{ cm}^{-1}$  and around  $3062\text{ cm}^{-1}$  respectively for the three CAS, which is slightly different from that of the MA CAS, where the values just fell to  $3174\text{ cm}^{-1}$  and  $3030\text{ cm}^{-1}$ . This is likely due to the acid O of VAL, forming a N-H-O synthon with the NH of NIC, which also displays reduction in stretching frequency, from  $1729\text{ cm}^{-1}$  to  $1717\text{ cm}^{-1}$  for the MA CAS, but further lowering to approximately  $1713\text{ cm}^{-1}$  for the other CASs. This downward shift was also observed by the NH bending vibrations due to this hydrogen bonding, from  $1615\text{ cm}^{-1}$  and  $1575\text{ cm}^{-1}$  to  $1603\text{ cm}^{-1}$  and  $1567\text{ cm}^{-1}$ . Both these bonds are presented in the VAL-NIC1 heterosynthon with N-H<sub>NIC-amide</sub>---O<sub>VAL</sub> and O-H---O<sub>NIC-amide</sub> bonding in the system, as demonstrated in [Figure 5.9 \(a\)](#). However, the peak assigned to asymmetric stretching vibration of the NH bond of NIC in the MA and SL CASs, remains partially unshifted, which indicates that only one NH bond binds to the C=O<sub>acid</sub> group at a time, whilst the other one perhaps exists freely. This lack of change is not noticed in the BM and SD CASs, where there is a decrease in the peak position by only  $22\text{ cm}^{-1}$ . This unbonded NH of NIC can then bond with another molecule of VAL which can lead to an excess of unbonded NIC and cause phase separation in all four formulations. Moreover, these free NH bonds in the CASs could bind to water molecules which may be present in the powders, thereby disrupting the stability of the co-amorphous systems, which is presented in the next chapter.

In all four CASs, the C=O<sub>amide</sub> of VAL undergoes a small increase of  $6\text{ cm}^{-1}$  to  $1603\text{ cm}^{-1}$  (merged with the NH bending vibrations), possibly due to the hydrogen bonding interactions with the NH of NIC. Additionally, the tetrazolyl NH peak of VAL at  $3455\text{ cm}^{-1}$  goes undetected across all the all CASs prepared by different processing techniques, which indicates that it has undergone hydrogen bonding interactions with the C=O<sub>amide</sub> of NIC, as indicated by its red shift, as presented in VAL-NIC2 heterosynthon in [Figure 5.9 \(b\)](#), as was observed in [Chapter 4](#).

In the last chapter, spectra of three heterosynthons were calculated and compared to the equimolar MA CAS. It showed that calculated spectrum of the three-molecule heterosynthon of 2 molecules of NIC and one molecule of VAL seemed to fit the experimental data of equimolar MA system better than that of the calculated spectra of two equimolar heterodimers. In [Figure 5.11](#), those calculated spectra are grouped together in a box labelled *Computational Studies* and stacked over the experimental spectra of the four CASs prepared by the four different methods. Just like the MA CAS, the spectra of the three other CASs show more similarities in intensities and shape to the DFT-calculated IR data of the trimolecular heterosynthon than close of the heterodimers, within

the range of 1600 to 1500  $\text{cm}^{-1}$ , where the hydrogen bonding takes place between the amide and acid groups of NIC and VAL respectively. This indicates the three molecular heterosynthon may be present in all equimolar CASs of VAL and NIC, irrespective of their method of preparation, where the larger API molecule binds with two smaller excipient molecules. This can lead to an excess of VAL existing freely within X-ray amorphous equimolar CASs, thereby generating inhomogeneity throughout the system, which can potentially destabilise the system and enable recrystallisation.

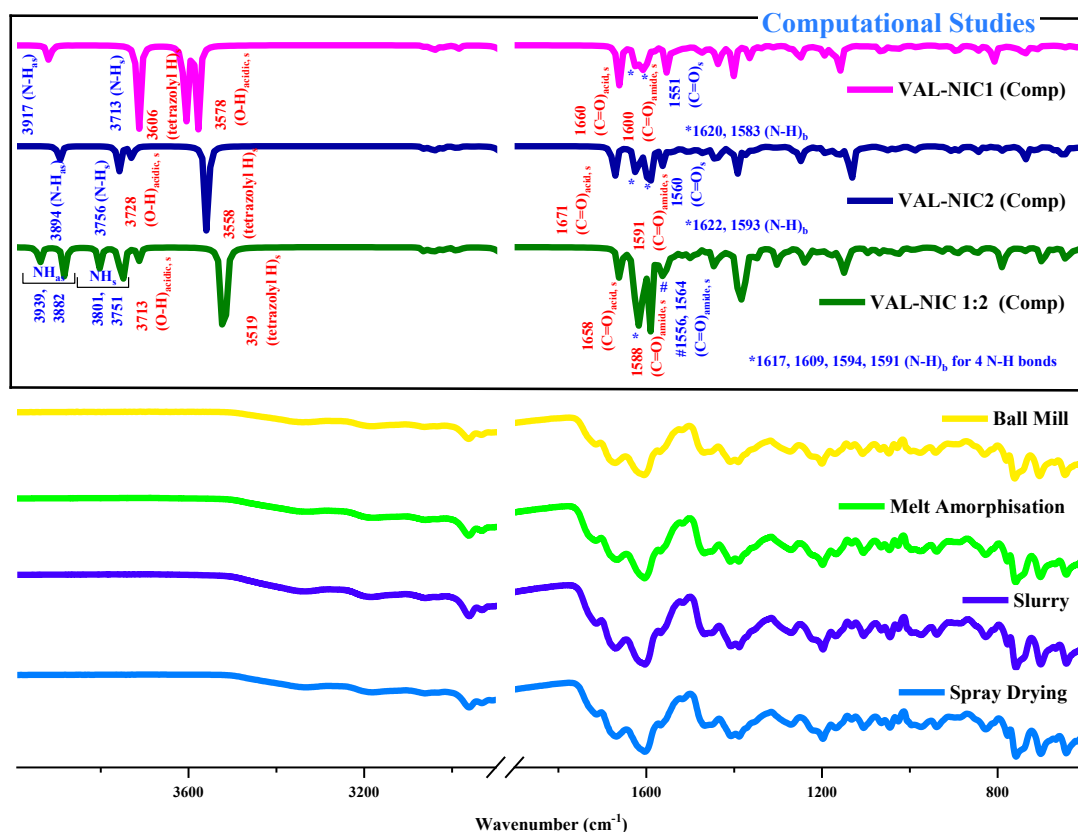


**Figure 5.11:** (In magenta, navy blue and green) Computational FT-IR spectra for equimolar co-amorphous systems of valsartan and nicotinamide and the VAL:NIC 1:2 system. Experimental FT-IR of the four CASs are stacked below the box labelled computational studies.

#### 5.3.4.2. Raman Spectroscopy

Raman spectroscopy has been employed to study non-polar interaction within the co-amorphous systems, something that the FT-IR does not readily detect.

The interaction involving carbonyl and NH groups can be observed between the range of 1800 to 1500  $\text{cm}^{-1}$ , as presented in Figure 5.12 and in Table 5.2. VAL (in red) shows three characteristic peaks at 1608  $\text{cm}^{-1}$ , 1583  $\text{cm}^{-1}$  and 1564  $\text{cm}^{-1}$  corresponding to  $\text{C}=\text{O}_{\text{amide}}$  stretching,  $\text{C}=\text{C}$  stretching and NH bending vibrations, respectively. The NIC

(in green) showed characteristic peaks at  $1580\text{ cm}^{-1}$  which corresponding to the C=C stretching, whilst  $1598\text{ cm}^{-1}$ ,  $1616\text{ cm}^{-1}$  and  $1679\text{ cm}^{-1}$ , where the first two correspond to the NH bending vibrations and the last peak corresponds to the C=O stretching vibrations.

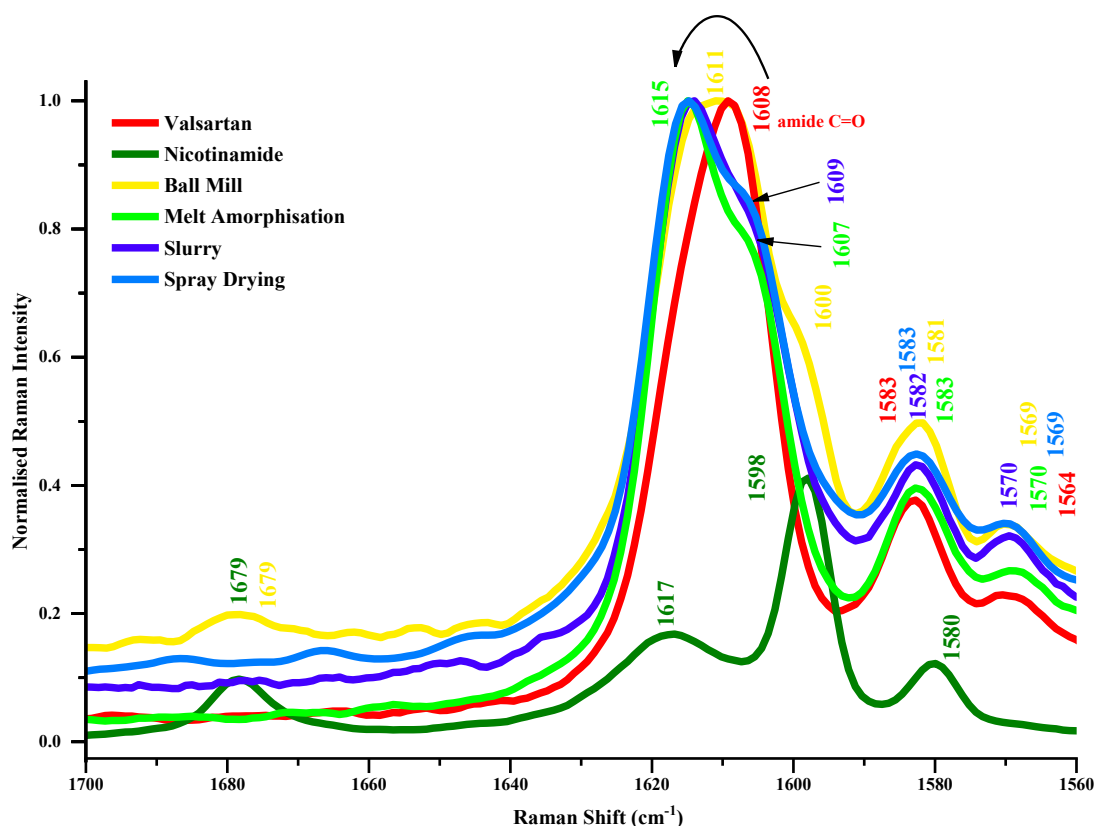


**Figure 5.12:** Raman spectra for as-bought VAL, NIC, and the co-amorphous formulations.

A change in the peak positions is observed in all the co-amorphous formulations, similar to the changes in the MA CASs presented in [Chapter 4](#). In an amide-acid heterosynthon, there is an increase in the amide carbonyl bond length (in NIC) as the carbonyl oxygen forms a H-bond with the hydroxyl H of VAL, thus increasing the C=O bond length of NIC, thus causing a lowering of frequency, making the peak undetectable in the co-amorphous systems. (232). It is observed that the C=O<sub>amide</sub> peak of NIC at  $1679\text{ cm}^{-1}$  merges with the background in [Figure 5.12](#) in all CASs except the BM CAS, where it is prominent. This is potentially due to the presence of NIC molecules existing freely within the amorphous dispersion, thereby solidifying the evidence of inhomogeneity in BM CAS. C=O<sub>amide</sub>-VAL stretching frequency increases to  $1615\text{ cm}^{-1}$  and  $1614\text{ cm}^{-1}$  from  $1608\text{ cm}^{-1}$  for the SD and SL CASs respectively, similar to that of the MA CAS, where an increase in the peak position by  $7\text{ cm}^{-1}$  has been observed. However, the C=O<sub>amide</sub> of VAL for the BM CAS is wider in comparison to that of VAL and the other CASs, undergoing a small increase of  $3\text{ cm}^{-1}$ , to  $1611\text{ cm}^{-1}$ , compared to that of other CASs. The

peak broadening suggests amorphisation of VAL within the CAS (256). However, this small increase and broadening could be due to the presence of bonded and unbonded VAL in the system, where the two peaks from both the VAL merge together to form a wider peak, with the peak intensity being highest at  $1611\text{ cm}^{-1}$ , providing another evidence of inhomogeneity in the CAS prepared in a mechanochemical method. The small peak at  $1564\text{ cm}^{-1}$  can be assigned to  $\text{N-H}_{\text{tetrazolyl}}$  bending, which increases to  $1570\text{ cm}^{-1}$  for MA and BM CASs and  $1569\text{ cm}^{-1}$  for SD and SL CASs. These shifts could be due the second bonding site which involves N-H of NIC bonding with  $\text{C=O}_{\text{amide}}$  of VAL and  $\text{C=O}_{\text{amide}}$  of NIC which bonds with  $\text{N-H}_{\text{tetrazolyl}}$  of VAL, a bonding that occurs in VAL-NIC2 heterosynthon as shown in the [Figure 5.9 \(b\)](#), same as the MA CASs in [Chapter 4](#).

**Table 5.2:** Experimental Values of Raman spectra for VAL, NIC, and the co-amorphous systems along with shifts ( $\Delta$ ) relative to VAL and NIC. The VAL and NIC values are in red and green respectively, mirroring the data in [Figure 5.12](#). Stretching and bending interactions are demoted by the suffices s and b respectively and UD indicates that the peaks go undetected.

| Components | Functional Groups                    | Systems ( $\text{cm}^{-1}$ ) |                                           |                                                       |                                           |                                           |
|------------|--------------------------------------|------------------------------|-------------------------------------------|-------------------------------------------------------|-------------------------------------------|-------------------------------------------|
|            |                                      |                              | MA CAS                                    | BM CAS                                                | SD CAS                                    | SL CAS                                    |
| VAL        | $\text{C=O}$<br>(amide) <sub>s</sub> | 1608                         | 1615, ( $\Delta=7$ ),<br>shoulder at 1606 | Broad peak at 1611 ( $\Delta=3$ )<br>shoulder at 1600 | 1615, ( $\Delta=7$ ),<br>shoulder at 1608 | 1614, ( $\Delta=6$ ),<br>shoulder at 1609 |
|            | $\text{C=C}_s$                       | 1583                         | 1570 ( $\Delta=-13$ )                     | 1569 ( $\Delta=-14$ )                                 | 1570 ( $\Delta=-13$ )                     | 1569 ( $\Delta=-14$ )                     |
|            | $\text{N-H}_b$                       | 1564                         | 1570 (merged with NIC) ( $\Delta=6$ )     | 1569 (merged with NIC) ( $\Delta=5$ )                 | 1570 (merged with NIC) ( $\Delta=6$ )     | 1569 (merged with NIC) ( $\Delta=5$ )     |

|     |                             |               |                                                    |                                                         |                                                     |                                                    |
|-----|-----------------------------|---------------|----------------------------------------------------|---------------------------------------------------------|-----------------------------------------------------|----------------------------------------------------|
| NIC | C=O<br>(amide) <sub>s</sub> | 1679          | UD                                                 | UD                                                      | 1677                                                | UD                                                 |
|     | N-H <sub>b</sub>            | 1617,<br>1598 | UD, 1606<br>(merged<br>with VAL)<br>( $\Delta=8$ ) | UD, 1611,<br>(merged<br>with<br>VAL)<br>( $\Delta=13$ ) | UD, 1608<br>(merged<br>with VAL)<br>( $\Delta=10$ ) | UD, 1606<br>(merged<br>with VAL)<br>( $\Delta=8$ ) |
|     | C=C <sub>s</sub>            | 1580          | 1570<br>( $\Delta=-10$ )                           | 1569<br>( $\Delta=-11$ )                                | 1570<br>( $\Delta=-10$ )                            | 1569<br>( $\Delta=-11$ )                           |

The peak shifting from 1598 cm<sup>-1</sup> of crystalline NIC to 1606 cm<sup>-1</sup> (a shoulder) in the amorphous form, was assigned to the vibration of the N-H bond (increase in bond length due the H-bonding, which increases the electron density at the N, causing an increase in bond angle, thus increasing bending vibration), a shift similar to that of the N-H<sub>tetrazolyl</sub> bending vibration of VAL. The peak at 1616 cm<sup>-1</sup> merges with the background, due to the hydrogen bonding interaction between the C=O<sub>amide</sub> of VAL and N-H of NIC. Furthermore, N-H is a heteronuclear bond, which is not Raman sensitive, making it easier for these peaks to go undetected as they produce weak Raman signals (266). Thus, the Raman data show presence of VAL-NIC2 heterosynthon across all four equimolar CAS formulations, irrespective of their mode of preparation.

The downward shift in peaks corresponding to C=C stretching vibrations, is the same as observed in the MA CASs in [Chapter 4](#), from 1583 cm<sup>-1</sup> to 1570 cm<sup>-1</sup> for VAL and 1580 cm<sup>-1</sup> to 1570 cm<sup>-1</sup> for NIC, indicate aggregation due to the formation of  $\pi$ - $\pi$  stacking interactions, between the aromatic rings of the two components (135). This suggests that the all VAL and NIC are undergo molecular mixing to produce CASs that exhibit  $\pi$ - $\pi$  interactions to stabilise the equimolar CASs, in addition to hydrogen bonding interactions, irrespective of how they were prepared.

From the Raman data, we observe that thermodynamical processing techniques (MA, SL, SD) produce similar data, with the bonding interaction similar to each other and have similar levels of homogeneity based on the spectroscopic data alone, but kinetically produced system i.e. the BM CAS is different. The hydrogen bonding

interactions which should be present within the CAS is present but the presence of unbonded components is also detected. This suggests that significant inhomogeneity is present in the BM CAS, as evidenced by thermal studies in section 5.3.2 and PLM images in section 5.5.3.1.

Like Chapter 4, FT-IR spectroscopy identified the polar hydrogen bonds between the VAL and NIC involving the amide group of NIC and acid group of VAL whilst Raman spectra identified the non-polar hydrogen bonding interaction between the amide group of VAL involving the carbonyl group, and tetrazolyl H of VAL with the amide group of NIC as well as  $\pi$ - $\pi$  interactions between aromatic groups of the two components. Furthermore, the findings of FT-IR and Raman spectroscopy suggest the presence of all three heterosynthons (VAL-NIC1, VAL-NIC2 and NIC-VAL-NIC, shown in Figure 5.9) in all the CASs prepared by different methods, just like it was present in MA CAS in the previous chapter.

### 5.3.5. Equilibrium Solubility

The equilibrium solubility of a compound in a solvent is defined as the maximum amount of the most stable solid form of that compound that can remain dissolved at a given volume of that solvent at a specific temperature and pressure (7, 267). The main goal of the formulation of a co-amorphous solid drug delivery system is improving solubility of poorly soluble API, in this case VAL. The equilibrium solubility plot for SC VAL, Am VAL and the CASs are shown in Figure 5.13 and tabulated in Table 5.3. The solubility of the VAL on its own, for both the semi-crystalline API and its amorphous form is 0.22 mg mL<sup>-1</sup>. Amorphisation of the drug alone did not enhance the solubility above that of the semi-amorphous drug.

Co-amorphisation of VAL with NIC, an excipient with a solubility of 1 g mL<sup>-1</sup>, significantly enhanced the solubility of VAL in the formulations (258). The BM CAS exhibits a 22-fold increase in solubility, with it being at 4.8 mg mL<sup>-1</sup> with the VAL solubility in the drug delivery system being 3.82 mg mL<sup>-1</sup>, a 17-fold increase. The MA and SL CASs have similar increase in the solubility in the binary system, to 6.05 and 6.1 mg mL<sup>-1</sup> respectively, which is around 28 times increase in the solubility over the as-bought VAL. The solubility of VAL within MA and SL binary system is 5.04 and 4.91 mg mL<sup>-1</sup> respectively, showing an approximate 22-fold increase in its solubility within the co-amorphous formulations. The highest solubility of a CAS was recorded by the SD

CAS at 7.04 mg mL<sup>-1</sup>, a 32-fold increase in solubility and VAL within the system exhibited a 26-fold increase in solubility at 5.67 mg mL<sup>-1</sup>.

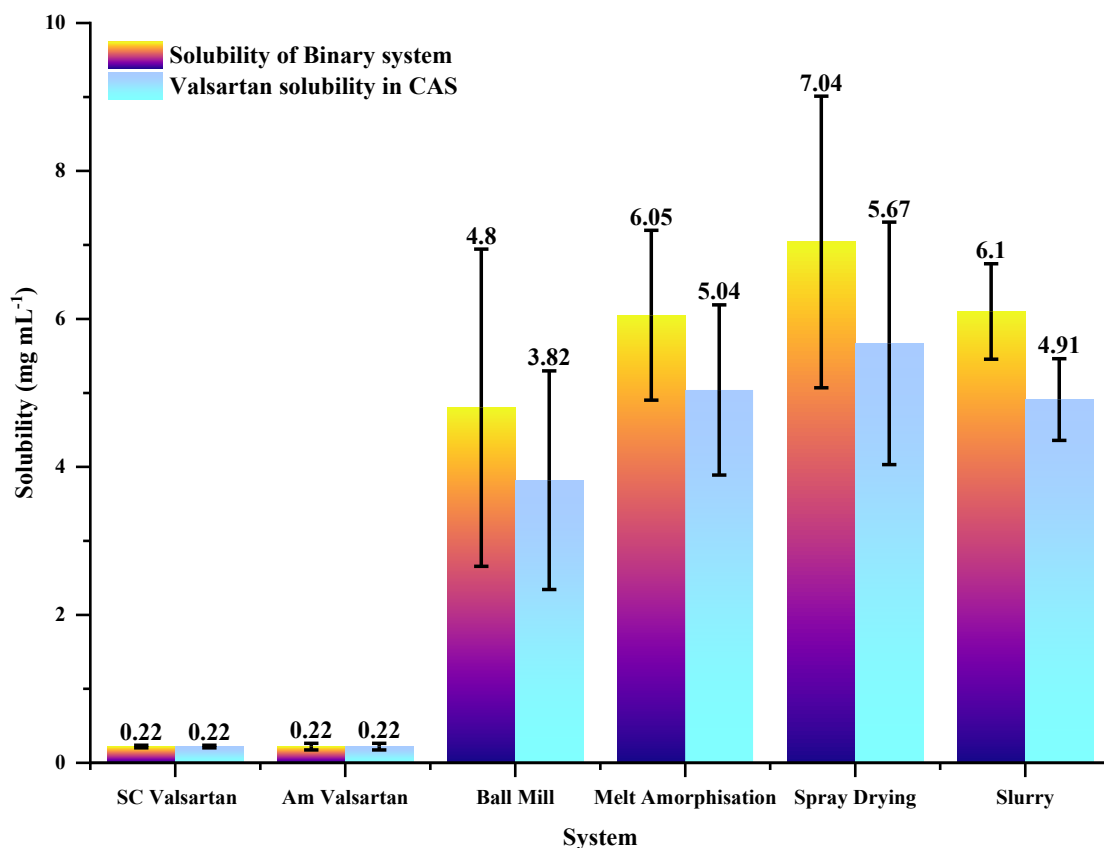


**Figure 5.13:** Equilibrium solubility values of VAL and its co-amorphous formulations in water prepared by different preparation methods. The bars in multicolour represent the solubility of the CASs as a whole and the bars in blue represent the solubility of the drug within the co-amorphous system. Each experimental data is an average of three repeats along with a standard deviation depicted by the error bars.

Aqueous solubility of the CASs and API improved significantly as NIC is a known hydrotrope (108). The hydrophilic group of NIC interacts with the water molecules, thereby disrupting the tetrahedral network of water, making “pockets” for the API to readily dissolve in the solvent as the hydrophobic end of the NIC is still bonded to that of the VAL, thereby driving the disintegration of the VAL particles and increasing the amount of VAL that can readily dissolve in water.

**Table 5.3:** Solubilities of SC VAL, Am VAL and co-amorphous formulations in water (pH 5.98) and phosphate buffer (PBS) (pH 7.4) at 25 °C. Each value is an average of three repeats along with a standard deviation.

|                    | Solubility (mg mL <sup>-1</sup> ) |                                        |                  |                                        |
|--------------------|-----------------------------------|----------------------------------------|------------------|----------------------------------------|
|                    | Water                             |                                        | Phosphate Buffer |                                        |
| Systems            | Binary system                     | Solubility of VAL in the binary system | Binary system    | Solubility of VAL in the binary system |
| SC Valsartan       | 0.22± 0.02                        | -                                      | 6.35±0.09        | -                                      |
| Am Valsartan       | 0.22± 0.05                        | -                                      | 6.06±0.80        | -                                      |
| Nicotinamide       | 1000                              | -                                      | 84.63±8.60       | -                                      |
| Ball Mill          | 4.80±2.14                         | 3.82±1.48                              | 5.94±2.78        | 4.96±1.92                              |
| Melt Amorphisation | 6.05±1.15                         | 5.04±1.04                              | 4.36±1.72        | 3.81±1.04                              |
| Spray Drying       | 7.04±1.97                         | 5.67±1.64                              | 3.86±1.08        | 3.40±0.67                              |
| Slurry Mixing      | 6.10±0.65                         | 4.91±0.55                              | 5.76±0.72        | 4.60±0.52                              |

Interestingly, the equilibrium solubility in PB solution, however, takes a downward trend, as reported in Table 5.3. The equilibrium solubility of the SC VAL in PBS (pH 7.4) is 6.35 mg mL<sup>-1</sup>. The Am VAL shows a slight decrease in the PB solubility, at 6.06 mg mL<sup>-1</sup>. This downward trend in PBS solubility was observed in the CASs as well. The system with highest PBS solubility was the BM CAS, at 5.94 mg mL<sup>-1</sup> and the solubility of VAL in that system is 4.96 mg mL<sup>-1</sup>, indicating a 22% decrease in the solubility of the drug in the CAS. SL CAS and MA CAS have reduced solubilities of 4.36 and 5.76 mg mL<sup>-1</sup> respectively, with the VAL solubility dropping to 3.81 and 4.60 mg mL<sup>-1</sup> respectively, demonstrating a 40% and 28% decrease in VAL solubility in PBS in these two respective systems. However, the SD CAS, which had the highest aqueous solubility, now has the lowest PBS solubility at 3.86 mg mL<sup>-1</sup>, and the solubility of the API in the binary system drops by 47% to 3.40 mg mL<sup>-1</sup>. This decrease in PBS solubility

of VAL in CASs could be due to solubility of NIC PBS, which is determined to be  $84.63 \pm 5 \text{ mg mL}^{-1}$ . The low solubility of NIC in PBS in comparison to its solubility in water, potentially negatively affects the PBS solubility of VAL and thereby reducing it. The enhancement of aqueous solubility of the CASs, along with the decrease in their solubilities in PB solution suggests that the solubilising capability of the excipient in the respective solvents plays a significant role in the overall CAS solubility, which ultimately influences the solubility of the API that is bonded to the excipient in the said CAS. Considering of the different solubilities of the CASs in both water and PBS, it can be presumed that the different preparation techniques lead to different intermolecular interaction within the systems, pertaining to the varying morphologies of the powders. The equilibrium (aqueous) solubility can simply be demonstrated in this manner: SD CAS > SL CAS > MA CAS > BM CAS > VAL (both SC and Am VAL).

Aragón-Aburto *et al* demonstrated that co-amorphisation enhances the solubility of glibenclamide, as it was insoluble in its crystalline and amorphous forms at low pH values pH ( $\leq 6.8$ ), and the solubility of both forms of the drug and its co-amorphous formulation increased with increasing pH ( $\geq 7.4$ ) (268). Similarly, Chen *et al* observed that only at pH 6.8, solubility of amorphous celoxib was 2.6 times more than that of its crystalline form and co-amorphisation with imipramine increased the crystalline API solubility by 64.6-fold; solubility at lower pH values was undetectable (269).

The data in [Figure 5.13](#) and [Table 5.3](#) reflect these reported findings as the solubility of SC and Am Val increase from  $0.22 \text{ mg mL}^{-1}$  in water at pH 5.98 to  $6.4 \text{ mg mL}^{-1}$  in PB solution at pH 7.4. This is expected as VAL is a weak acid and it is not easily ionised at lower pH values, and the solubility increases as the pH increases. Solubility of VAL in water has been reported to be approximately  $0.19 \text{ mg mL}^{-1}$ , whereas for at pH 7.4 it is  $8.5 \text{ mg mL}^{-1}$ , with the solubility values being considerably low at lower pH (10, 34, 260). In a buffered solution of pH 7.4 (a value significantly higher than both the  $pK_a$  values of VAL), VAL solubility increases due to the formation of the di-anionic conjugate base, which binds well with water molecules, thereby solvating VAL easily (255).

The data in [Figure 5.13](#) and [Table 5.3](#) show that the solubility of NIC decreases from  $1000 \text{ mg mL}^{-1}$  in water at pH 5.98 to  $84.6 \text{ mg mL}^{-1}$  at pH 7.4. NIC is considered to be in a neutral, highly soluble form with no significant pH-dependency variation between acidic and physiological pH levels (such as pH 5.0 and pH 7.4) (270). NIC solubility in water is reported to be  $1000 \text{ mg mL}^{-1}$  at  $20^\circ\text{C}$  and Sjöberg *et al* reports this to remain as

high as 463 mg mL<sup>-1</sup> in pH 7.4, similar to the trend presented here in the current data (258, 270).

The solubilities across the binary CASs reported in Figure 5.13 and Table 5.3 are similar at pH 5.98 with values averaging at 5.99 mg mL<sup>-1</sup>, assumed to be due to the hydrotropic effect of NIC enhancing the solubility of VAL in water. The solubility of the VAL in the co-amorphous systems is around 4.98 mg mL<sup>-1</sup>, consistent with the values reported by Turek *et al* (Table 4 says 3-5 mg mL<sup>-1</sup> for slurry and ball milled CASs) (10). This is also consistent with wider studies that report co-amorphisation enhances API solubility, by a factor of at least 1.2, regardless of pH values (10, 268, 269).

At pH 7.4, the solubility of the CASs is between 3 to 6 mg mL<sup>-1</sup> despite VAL's enhanced solubility at the higher pH values. This could be due to the measured drop in NIC solubility at the higher pHs or due to a combination of changes in both the VAL and NIC solubilities. In addition, there will be an increased quantity of ions in the PB solution compared to the water dispersion due to the presence of buffer salts, these ions will interact with the water molecules to reduce the amount available to solubilise VAL i.e. lowering its apparent solubility at this pH. Regardless of the precise reason, the solubility of the binary system as a whole is marginally reduced at the higher pH and becomes similar to that of SC or Am VAL at that pH. Turek *et al* measures a small increase in solubility of the VAL-NIC CASs in PB solution, around 10.5 mg mL<sup>-1</sup>. More generally, Lin *et al* observed that there had been no changes in the solubility of the binary systems of luteolin with arginine and lysine at pH 7.4, despite demonstrating a significant increase in solubility in water, and attribute this to charge neutralisation in this co-amorphous salt system (271). Additional studies are required to understand this reverse pH-dependent solubility exhibited by the VAL-NIC systems, which is beyond the scope of this PhD.

The equilibrium solubility of the drug in the co-amorphous formulations in water has significantly increased but has fallen in phosphate buffer. This confirms that co-amorphisation improves the aqueous solubility of VAL and has no positive impact in its solubility in phosphate buffer.

### 5.3.6. Conventional Dissolution Studies

Dissolution studies is a physiological test which forms a significant part of the synthesis of drug dosage. It is crucial to assess drug product quality in terms of stability and drug release. In the early stages of development, investigation of in-vitro dissolution

characteristics of an API helps in co-former selection for the development of new formulations or improvement of formulations in circulation (272). Here, a conventional dissolution experiment has been conducted to measure how these formulations released the active ingredient over time as a whole ( $\mu\text{g mL}^{-1} \text{sec}^{-1}$ ), contrary to the intrinsic dissolution method, where the dissolution rate of the drug is evaluated by maintaining a constant surface area that is exposed to the dissolution media ( $\mu\text{g min}^{-1} \text{cm}^{-1}$ ).

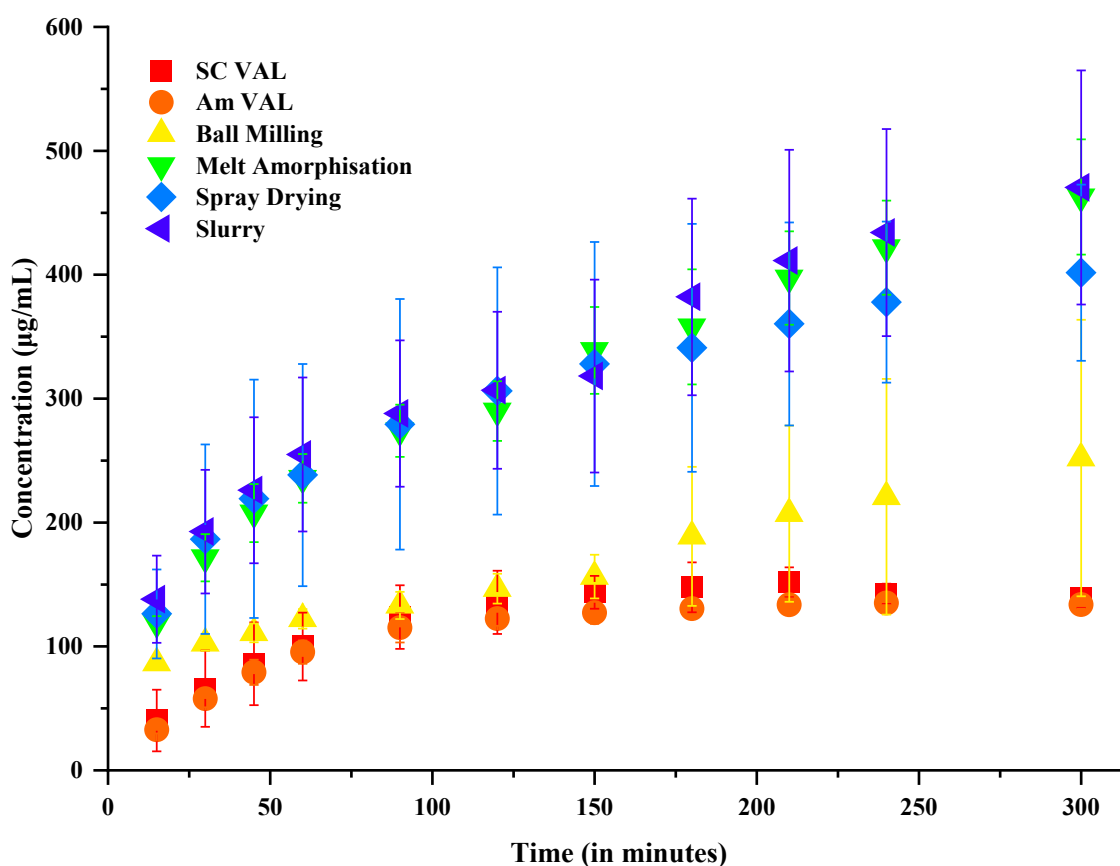


**Figure 5.14:** Dissolution profiles of SC VAL (red), Am VAL (orange), Ball Milled CAS (yellow), Melt Amorphised CAS (green), Spray Dried CAS (blue) and Slurry Mixed CAS (purple). The dissolution study was performed in Milli-Q water (pH 5.98) at 37 °C. Each experimental data point is an average of three repeats along with a standard deviation depicted by the error bars.

The dissolution rates (DR) for VAL, amorphous VAL and the CASs were conducted under sink conditions in Milli-Q water (pH 5.98) at 37 °C for 300 minutes. **Error! Reference source not found.** demonstrates the dissolution profile of the semi-crystalline VAL and amorphous VAL as well as the VAL-NIC CASs prepared by the four methods. At each time point, the amount of solute dissolved for all the CASs was higher than that of semi-crystalline VAL and amorphous VAL. To calculate the DR, the drug release per volume of solvent ( $\mu\text{g mL}^{-1}$ ) was plotted against time. The DR ( $\mu\text{g mL}^{-1} \text{min}^{-1}$ )

was then obtained by the linear fit of the dissolution data points, whilst assuming that the CASs were single-phase homogeneous systems.

15 minutes into the experiment, about  $40.2 \mu\text{g mL}^{-1}$  of SC VAL dissolved in the solvent and showed an increase to  $138.8 \mu\text{g mL}^{-1}$  after 300 minutes of the experiment. It is expected that the amorphous form of the drug would demonstrate higher dissolution compared to its semi-amorphous counterpart but that was not the case for Am VAL. About  $32.6 \mu\text{g mL}^{-1}$  of Am VAL dissolved within quarter of an hour and did not show any significant increase throughout the experiment in comparison to its semi-crystalline counterpart, only dissolving  $133.8 \mu\text{g mL}^{-1}$  after 300 minutes. Amongst all the four co-amorphous formulations, the SL CAS demonstrated the highest dissolution whereas the BM CAS demonstrated the lowest dissolution. The SL CAS dissolved the highest in 15 minutes, in comparison to all the other CASs and the API (both semi-crystalline and amorphous VAL), at  $138.1 \mu\text{g mL}^{-1}$  into the first quarter of an hour of the experiment, the amount of solute that was dissolving gradually increasing to  $470.5 \mu\text{g mL}^{-1}$  after 300 minutes, which is a 3.4-fold increase over that of the semi-crystalline API. The SD and MA CASs, have relatively similar dissolution profiles, starting at  $126.2 \mu\text{g mL}^{-1}$  and  $119.1 \mu\text{g mL}^{-1}$  at 15 minutes for SD and MA CASs, respectively. The amount of MA and SD CASs dissolved in solvent increases gradually in a manner similar to each other till 210 minutes. After that, the MA CAS demonstrates increased dissolution of solute at  $397.3 \mu\text{g mL}^{-1}$  dissolved in 210 minutes whereas  $360.3 \mu\text{g mL}^{-1}$  of SD CAS dissolved in the same duration. By 300 minutes, the MA and SD CASs reached values of  $462.8 \mu\text{g mL}^{-1}$  and  $401.7 \mu\text{g mL}^{-1}$ , respectively. As mentioned before, BM CAS demonstrated the poorest dissolution amongst all the CASs, dissolving only  $86.6 \mu\text{g mL}^{-1}$  at 15 minutes, maintaining the trend for the rest of the experiment, after which it shows a slight increase in dissolution and finishes at  $252.0 \mu\text{g mL}^{-1}$  after 300 minutes.

The dissolution rate (DR) of the drug and CASs can be determined from the dissolution profiles. **Error! Reference source not found.** and **Error! Reference source not found.** demonstrates the DR values of the SC VAL, Am VAL and co-amorphous formulations in in Milli-Q water (pH 5.98) at  $37^\circ\text{C}$  at 300 minutes. The Am VAL dissolved at the same rate as its semi-crystalline counterpart, with the rates being  $0.42$  and  $0.41 \mu\text{g mL}^{-1} \text{ min}^{-1}$ , respectively. The DR, improved with the co-amorphisation strategy, with the highest DR was for MA CAS at  $1.37 \mu\text{g mL}^{-1} \text{ min}^{-1}$ , an 3.3-fold increase

in comparison to SC VAL, followed by the SL CAS and SD CASs at 1.29 and 1.07  $\mu\text{g mL}^{-1} \text{min}^{-1}$  respectively, which is 3.2 and 2.6 times increase with respect to the SC VAL. However, BM CAS has the poorest DR out of all four CASs, with an DR of 0.53  $\mu\text{g mL}^{-1} \text{min}^{-1}$ , 1.3-fold increase in DR with respect to SC VAL. This small increase in comparison to other CASs, suggests that the two components may be amorphous within the system but are not miscible with each other, i.e., existing in amorphous state but not bonded together as evidenced by PLM and spectroscopic studies, although the singular glass transition event would suggest that the system exists in single amorphous phase. This presence of a single phase along with the lack of bonding between the two components and some small-scale crystallinity from NIC (from PLM images in [Figure 5.5](#)) attributes to the inhomogeneity within the BM CAS, which ultimately affects its performance.

**Table 5.4:** Dissolution rates of SC VAL, Am VAL and co-amorphous formulations in water (pH 5.98) at 37.5 °C. Each value is an average of three repeats along with standard deviation.

| Systems                    | Intrinsic Dissolution Rate ( $\mu\text{g mL}^{-1} \text{min}^{-1}$ ) in water |
|----------------------------|-------------------------------------------------------------------------------|
| Semi-Crystalline Valsartan | 0.41±0.04                                                                     |
| Amorphous Valsartan        | 0.42±0.02                                                                     |
| Ball Mill                  | 0.53±0.03                                                                     |
| Spray Drying               | 1.07±0.07                                                                     |
| Slurry Mixing              | 1.29±0.07                                                                     |
| Melt Amorphisation         | 1.37±0.10                                                                     |

Co-amorphisation augmented the equilibrium solubility for the poorly water-soluble VAL and the same trend is followed in the dissolution studies, as the high aqueous solubility of NIC helps with the faster disintegration of VAL. The SD and SL CASs have higher solubility than MA CAS, but DR does not follow this trend for these three systems, SL CAS at 1.29  $\mu\text{g mL}^{-1} \text{min}^{-1}$  (3.1-fold increase) and SD CAS at 1.07  $\mu\text{g mL}^{-1} \text{min}^{-1}$  (2.6-fold increase), demonstrating that MA CAS has the highest DR. However, co-amorphisation does not augment the DR of the CAS prepared by the mechanochemical technique. Despite the BM CAS showing almost a 22-fold increase in solubility in comparison to the semi-crystalline drug, the DR values do not reflect that, where the binary formulation has an DR of 0.53  $\mu\text{g mL}^{-1} \text{min}^{-1}$ , which is just 1.3 times of that of the

SC VAL. Dissolution studies indicated that the rate at which a VAL-NIC system dissolves is not entirely dependent on their equilibrium solubility. This disparity in the dissolution profile and equilibrium solubility of the BM CAS can be an outcome of the inhomogeneity existing within the system, where the two components are existing in amorphous form but are unbonded to each other.

### 5.3.7. Pair Distribution Function (PDF)

The methods of powder diffraction are commonplace for structural analysis of materials. The PDF patterns of [Figure 5.15](#) give the distance distribution of atomic pairs through the structure of the CASs. This assists in identifying different interaction between the atoms, both polar and non-polar interactions which influences the structural ordering.

From [Figure 5.1](#), suggests that all the CASs are X-ray amorphous. However, [Figure 5.15](#) demonstrates (via X-ray PDF analysis) that is not the case. First, we have the SC VAL and Am VAL, where these two patterns are similar to each other as amorphisation does not change the structural integrity of the semi-crystalline VAL much, as seen in [Figure 5.15 \(a\)](#). The pattern of NIC is significantly different from that of VAL (in both forms) due to its crystalline nature. The peaks for the semi-crystalline and amorphous forms of VAL are present up to  $\sim 10$  Å whereas the peaks for crystalline NIC continue well beyond 15 Å, thus highlighting the difference in the short-range order of the drug and the long-range order of the excipient.

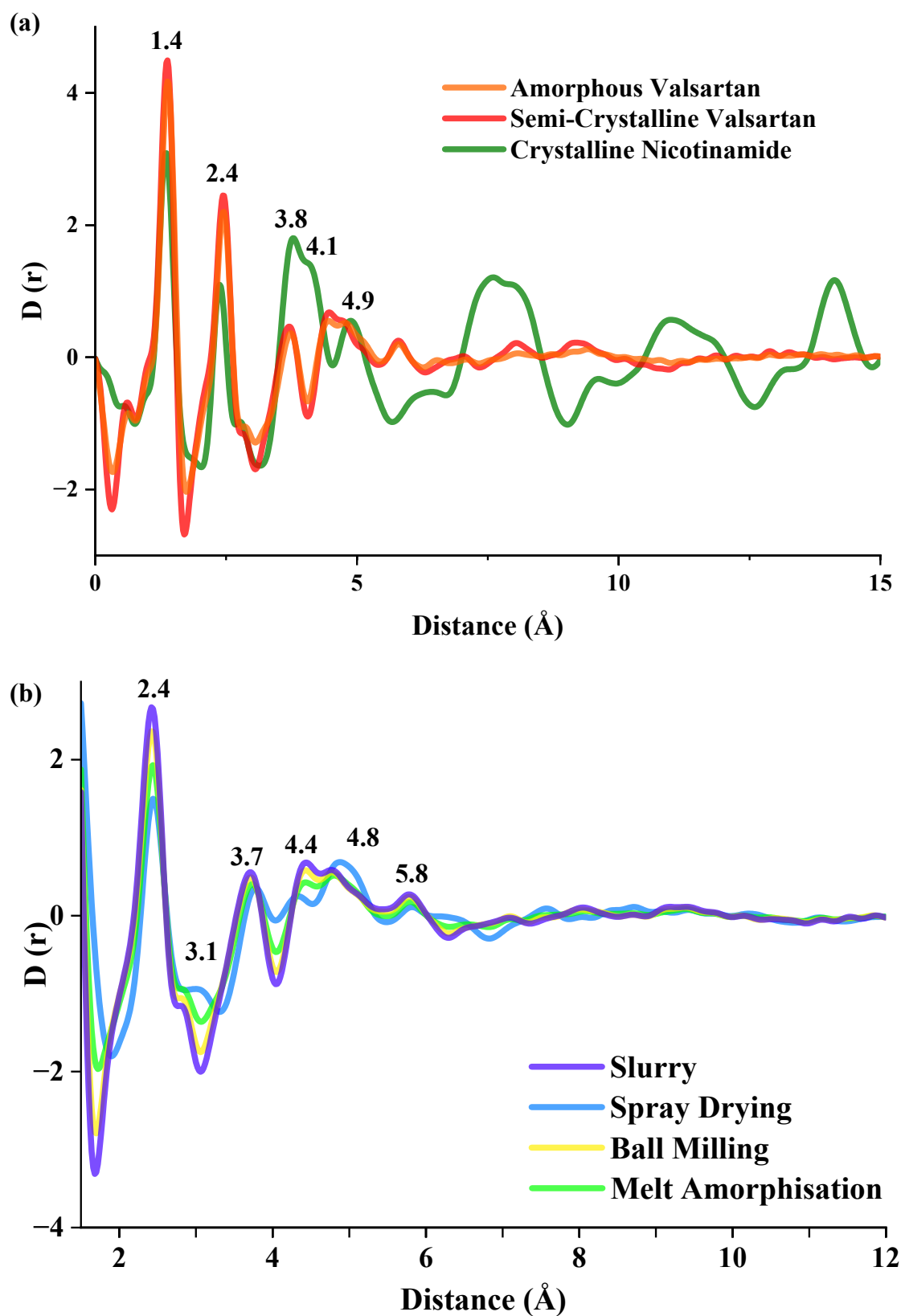


**Figure 5.15:** (a) Experimental PDF of the pure components, VAL and NIC and Am VAL (b) Experimental PDF of the co-amorphous formulations.

**Table 5.5:** Geometrical parameters involving the hydrogen bonding intermolecular interaction between the two components in the heterosynthons designed and optimised in GaussView 6.1.1.

| <u>System</u>      | <u>D–H···A</u>                                  | <u>H···A[Å]</u> | <u>D···H[Å]</u> | <u>D···A[Å]</u><br><u>(calculated)</u> |
|--------------------|-------------------------------------------------|-----------------|-----------------|----------------------------------------|
| <b>VAL/NIC1</b>    | O–H <sub>acid</sub> ···O <sub>amide</sub>       | 2.079           | 0.977           | 3.056                                  |
|                    | N–H <sub>amide</sub> ···O <sub>acid</sub>       | 2.082           | 0.996           | 3.078                                  |
| <b>VAL/NIC2</b>    | N–H <sub>tetrazolyl</sub> ···O <sub>amide</sub> | 2.061           | 1.014           | 3.075                                  |
|                    | N–H <sub>amide</sub> ···O <sub>amide</sub>      | 2.212           | 0.993           | 3.205                                  |
| <b>NIC-VAL-NIC</b> | O–H <sub>acid</sub> ···O <sub>amide</sub>       | 2.079           | 6.35            | 8.429                                  |
|                    | N–H <sub>amide</sub> ···O <sub>acid</sub>       | 2.207           | 0.989           | 3.196                                  |
|                    | N–H <sub>tetrazolyl</sub> ···O <sub>amide</sub> | 2.012           | 1.014           | 3.026                                  |
|                    | N–H <sub>amide</sub> ···O <sub>amide</sub>      | 2.154           | 0.991           | 3.145                                  |

The bond lengths present between multiple atom pair has been calculated by using the Crystallographic Information Files (cif files) present in CCDC database, namely NICOAM and KIPLIG for NIC and VAL, respectively. For both the systems, the intramolecular bond distances atomic pairs are within the range of 0.9 to 1.5 Å. Based on the heterosynthon models prepared in Chapter 4, shown in Figure 5.9, the bond lengths of the bonding sites have been tabulated in Table 5.5. All the intermolecular hydrogen bonding interaction take place within 2.2 Å for the heterodimers. However, some interactions taking place within the three molecular heterosynthon exceed beyond 6.5 Å, a region beyond which no peaks can be detected in the PDF pattern of any of the CASS in Figure 5.15 (b).

The lack of long-range crystallinity beyond 6.5 Å, i.e. peaks of high intensity, suggests the lack of the three-molecule heterosynthon, as the bonding interactions between exceeds 6 Å, in the NIC-VAL-NIC acid-amide bonding interaction of the three-molecule heterosynthon. This high distance could be due to the structural constrains, and such a high distance between the two bonding atoms would be weak and disrupt the

heterosynthon. Even if there is any such heterosynthon in the CASs, the possibility of its existence is quite low, thus making its existence only a theoretical possibility.

The four co-amorphous formulation produces PDF patterns which appear to be amalgamation of both the components, as presented in [Figure 5.15 \(b\)](#). Significant differences in the structural ordering can be observed between 1.7 to 5 Å. The patterns of the CASs start reflecting that of VAL, potentially due to their amorphous nature. This trend continues without any change for all the CASs except that of SD CAS. Instead of a at 1.7 Å, SD CAS demonstrates a shift in the peak to 1.9 Å, a value close to that of NIC. Furthermore, the PDF pattern of the SD CAS has a peak at 3.1 Å, which is not reflected by neither of the starting components, presented in [Figure 5.15 \(a\)](#). Additionally, the peak around 4.8 Å, reflected by all the CASs as well as the starting components, varies in position and intensity for the SD CAS from all the other systems and has a value higher than that of crystalline NIC itself. This deviation is indicative of a difference in structural framework of the SD CAS from the other systems prepared by different methods. This could also be due to the SD CAS being obtained from a solvent mixture of ethanol and water, which may have led to formation of a structure that has trapped water or ethanol molecule or both, bonded to both the starting components.

Beyond 8 Å, no long-range crystallinity can be detected due to the lack of atomic coherence for all the formulations indicating that they are predominantly amorphous with the presence of low levels of crystallinity. Despite the low levels of crystallinity, there is a distinction in the pattern of SD CAS to the other 5 amorphous systems. From 6.3 to 8.7 Å, the SD pattern has peaks and troughs that does not match the other CASs or both the VAL forms, further supporting the claim that the SD CAS has a different structural framework than rest of the binary systems and the pure components.

#### 5.4. Conclusion of Chapter 5

The aim of this chapter was to investigate the impact of four different processing techniques namely melt amorphisation, ball milling, spray drying and slurry mixing, on the CASs, upon the identification of the optimal VAL-NIC composition, in terms retained crystallinity along with performance of these binary formulations.

pXRD demonstrates the formation of X-ray amorphous systems by all four preparation techniques. No long-range crystallinity can be detected from the PDF patterns of all the binary formulations which indicates that the systems are essentially amorphous

with the presence of small amounts of crystallinity. However, there is a distinction in the PDF pattern of SD CAS from the other 5 systems (the other 3 CASs and the two forms of VAL) because the pattern of SD CAS does not match the other CASs or both forms of VAL, suggesting that it has a different structural framework than rest of the binary systems and the pure components, potentially due to the presence of solvent molecules trapped in the SD CAS, thus altering its short-range order from the other systems. This difference in pattern suggests that the different preparation modes affect the structural packing of the co-amorphous systems.

PLM detected phase separation in the BM and MA CASs, where NIC had undergone recrystallisation, suggesting inhomogeneity. Little to no birefringence was observed in the SL CAS. The SD CAS, prepared by a solvent mixture of water and ethanol, had very low levels of crystallinity, as evidenced by only some birefringence. Thus, solvent-based CASs appeared to be most homogeneous, due to removal of solvent from a uniform solution of the CASs. SEM images demonstrated that the as-bought components and their amorphous mixture have higher agglomeration and surface roughness whereas all the co-amorphous formulations appear similar and have a smoother surface, regardless of how they were prepared.

TGA indicates that the systems prepared by solvent evaporation reflected small mass loss which can be attributed to trapped ethanol in addition to moisture present in the system. *tmDSC* indicates the formation of a single-phase system, evidenced by the presence of a single glass transition event in each CAS on their second heating cycle. However, in the first heating cycle melting endotherms were present only in the MA and BM CASs indicating recrystallisation in both systems and amorphous-amorphous phase separation occurs only in the MA CAS.

FT-IR and Raman spectroscopy provide a comprehension of the interaction between the two components prepared by different techniques, by identifying the heterosynthons that stabilises these CASs, namely VAL-NIC1 and VAL-NIC2, and both can exist in co-amorphous formulation, irrespective of the method they were prepared with. The Raman data provided more evidence of inhomogeneity in the BM CAS as the C=O peak of NIC does not go undetected unlike the other CASs as well as the peak broadening of the C=O<sub>amide</sub> of VAL, indicating the presence of freely existing NIC and VAL molecules in the amorphous BM CAS. The experimental FT-IR data of the CASs is a closer match to the DFT-calculated IR data of the three-molecule heterosynthons (as

seen in [Chapter 4](#)), which would suggest that three-molecule heterosynthon can exist in the CASs. However, the formation of a three-molecule heterosynthon is only a theoretical possibility as PDF data does not find any evidence of its existence, thus ruling it out.

Solubility and dissolution studies support the claim that the co-amorphisation strategy significantly augments the aqueous solubility of VAL, as just amorphisation of the API did not enhance its performance. CASs prepared by solvent evaporation methods possess the highest solubility whereas mechanochemically prepared CAS has the lowest solubility, indicating that the mode of preparation affects the performance of the CASs. It was observed that solubility of the CASs is driven by the solubilisation capabilities of excipient. As the solubility of NIC is considerably high in water but moderate in PBS, the CASs follow the same trend, with enhancement of solubility in water and diminishment of solubility in PBS. The CASs prepared by thermodynamic disarrangement (MA, SD, and SL CASs) demonstrated higher dissolution rate than the API, possibly due to higher miscibility between the components. The dissolution profile of BM CAS lies between the other 3 CASs and the two forms of VAL, with a dissolution rate close to that of the drug. This suggests that in the BM CAS, some VAL molecules may exist in amorphous state but not bonded with NIC, thus lowering the overall rate, suggesting that the inhomogeneity of the BM CAS ultimately adversely affects its performance.

Overall, the CASs prepared by solvent evaporation give the best performance in terms of equilibrium solubility and dissolution, with little to no detectable crystallinity when freshly prepared compared to the other methods, thereby highlighting the impact of processing on the co-amorphous formulations, thus fulfilling the aim of this chapter.

## Chapter 6. Influence of moisture on the performance of co-amorphous systems prepared by various techniques

### 6.1. Chapter Overview

This chapter investigates the effects of moisture on equimolar co-amorphous (CAS) drug delivery systems of valsartan (VAL) and nicotinamide (NIC) prepared by four different techniques, to test their stability against phase separation and recrystallisation, especially its effects on their glass transition temperatures ( $T_g$ s) as it has been reported that water can act as a plasticiser on these systems, by storing them under varying humidity conditions, as described in section 3.4.5 of Chapter 3 (184, 254). Thermal investigations into the phase separation within CASs were conducted by identifying the occurrence of multiple  $T_g$ s as well as reoccurrence of melting point endotherms. pXRD and PLM have been crucial in identifying the crystallinity of the systems every two weeks for up to 32 weeks. Moisture adsorption was detected by thermal and spectroscopic analyses, suggesting the possibility of amorphous hydrates. Phase separation in the form of nucleation of NIC as well as amorphous-amorphous phase separation (AAPS) indicate that these systems are unable to maintain their single-phase homogeneous state under high humidity, even at room temperature. Furthermore, the presence of amorphous hydrates, which disrupt the bonding interaction between VAL and NIC has a detrimental effect on the equilibrium solubility of these binary systems as the solubilising effect of NIC diminishes, and the solubility of all four systems falls to the same absolute value after only four weeks of storage at 85% RH, indicating that equilibrium solubilities of co-amorphous systems stored at high humidity are independent of recrystallisation tendencies as well as their mode of preparation. Systems stored at lower relative humidities did not demonstrate any substantial changes in crystallinity nor solubility but did undergo phase separation. The drug on its own, demonstrated poor aqueous solubility before and after ageing studies compared to the CASs which demonstrates that co-amorphisation remains a useful technique to enhance the solubility of an otherwise poorly soluble drug.

### 6.2. Introduction

In Chapter 5, equimolar co-amorphous systems of VAL and NIC were prepared by four different methods, namely melt amorphisation, ball milling, spray drying and slurry mixing.

Recrystallisation would nullify the solubility advantages of co-amorphous form products, thus making avoidance of recrystallisation during storage after preparation necessary. Storage in a moist environment has been reported to have an obvious effect on accelerating recrystallisation of amorphous drug delivery systems (96, 103, 273, 274). With regards to the performance of drug delivery systems, it has been observed that adsorbed moisture has a negative impact on its dissolution (274, 275). Therefore, it is of special importance to comprehend the underlying mechanisms of crystallisation of amorphous solids and find the effective approach to maintain physical stability. Thus, it is essential to explore the mechanism of moisture-induced amorphous-amorphous phase separation (AAPS) and recrystallisation and their influence on the solubility of the CASs.

Crystallisation is a two-step process of nucleation and growth, both of which have different kinetics. According to the classic nucleation theory (CNT), nucleation is the first step in the formation of a new phase in a first order transformation process such as crystallisation, which can be initiated from an already present metastable state formed from a second order transformation such as a glass transition. The formation of this new phase is influenced by several factors such as molecular mobility, temperature, and humidity. Nucleation being a stochastic process, can occur at different times within the system. For a metastable, inhomogeneous co-amorphous system prepared from a crystalline excipient and a semi-amorphous API, it is thought that phase separation might occur in the form of recrystallisation.

As mentioned previously, there is still a lot to be learnt about how different preparation routes affect long-term stability against recrystallisation, phase separation and performance of these co-amorphous systems. Drug delivery systems in their amorphous states are metastable and are expected to be sensitive to environmental humidity, thereby affecting stability and performance significantly during storage. The aim of this chapter was to evaluate the storage stability of the co-amorphous systems as well as the drug in both its semi-crystalline and amorphous state, by assessing the moisture uptake and observing the changes in the crystallinity and solubility under different relative humidity (RH), when stored at 85% and 25% RH at ambient temperature conditions.

### **6.3. Test of stability: Ageing Study**

The binary co-amorphous systems were tested against two different relative humidities of 85% and 25%. The humidity chamber with 85% RH was prepared by keeping a supersaturated salt solution of potassium chloride (KCl) inside a desiccator.

The systems were also placed in a desiccator with silica gel within which the relative humidity was 25%. The different RHs were measured using hygrometers. The ageing study has been carried out until recrystallisation was observed; the study was stopped as soon as all the co-amorphous systems displayed crystallinity by pXRD.

## **6.4. Result and Discussion**

### **6.4.1. Case study I: Valsartan (Semi-crystalline and amorphous)**

Amorphous Valsartan (Am VAL) was prepared from the semi-crystalline (SC) VAL by the melt amorphisation method, where it was heated up to 150 °C, and rapidly cooled to room temperature. It appeared to form into a glassy and transparent state which returns to being a white loose powder upon extraction. After 32 weeks of ageing under high humidity of 85% as well as for the low humidity of 25%, it maintains its powdery state, indicating no change in its physical properties.

The physical state of the SC VAL has also remained unchanged. It started as a loosely packed white powder and it remained as such, after 32 weeks in both high and low humidities.

#### **6.4.1.1. Powder X-Ray Diffraction (pXRD)**

The SC VAL is a semi-crystalline white powder, with pXRD peaks at 5.6° and 17.6°. [Figure 6.1 \(a\)](#) and [\(b\)](#) demonstrates the XRD patterns of SC and Am VAL stored in 25% RH respectively over a period of 32 weeks. At the low relative humidity, both SC and Am VAL maintained their physical state through the entire 32-week period of ageing.

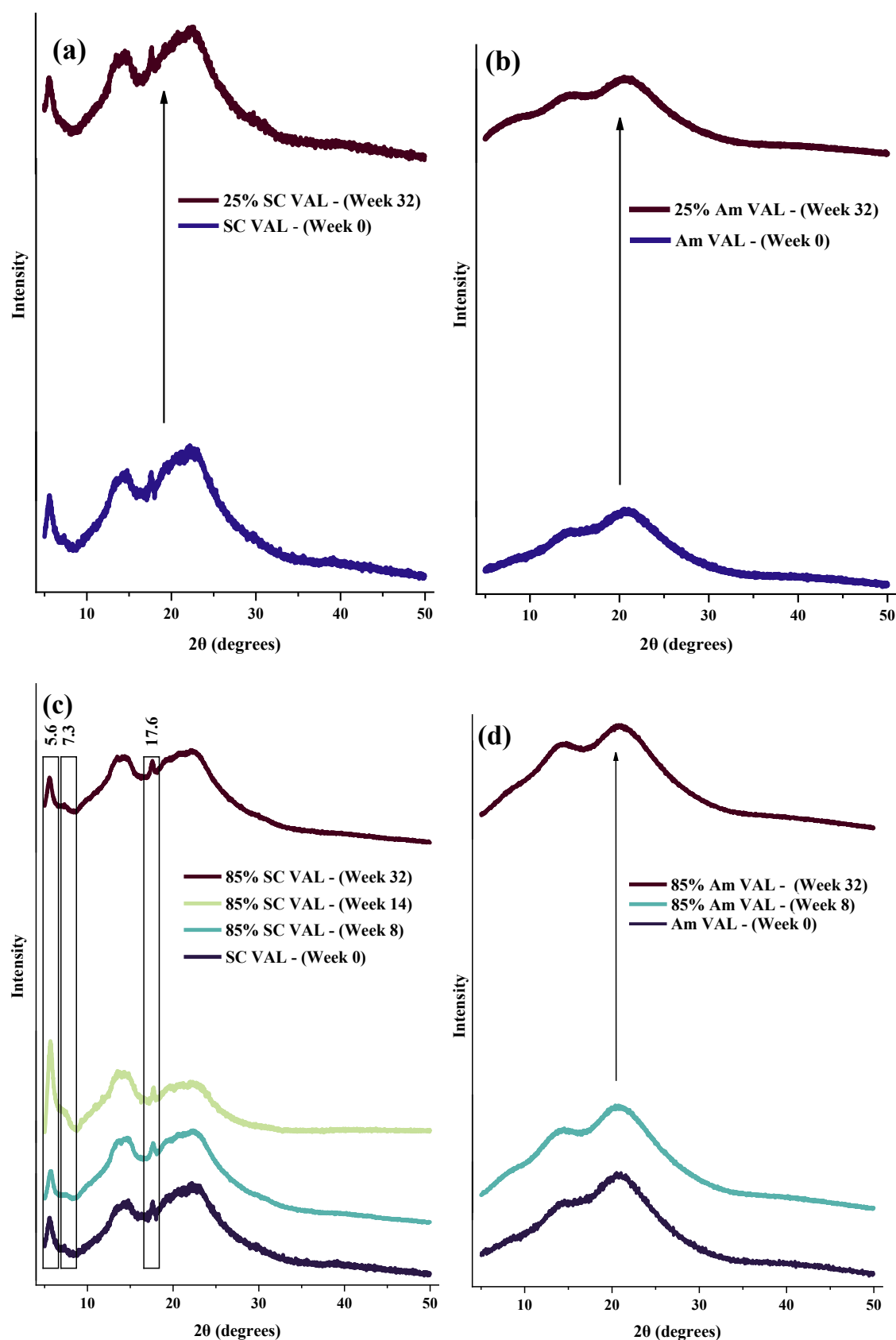


**Figure 6.1:** (a) and (b) PXRD patterns of SC VAL and Am VAL stored at 25% RH respectively at weeks 0 and 32. (c) and (d) PXRD patterns of SC VAL and Am VAL stored at 85% RH respectively at weeks 0 and 32.

Figure 6.1 (c) and (d) demonstrates the XRD patterns of SC and Am VAL stored in 85% RH respectively over a period of 32 weeks. Over the full 32 weeks of storage at 85% relative humidity, the system has not changed much, with no additional peaks appearing. There is a small peak at  $7.3^{\circ}$  (which is often undetectable) that gets slightly sharper with time. However, when stored at 25% RH, the peaks at  $5.6^{\circ}$  and  $17.3^{\circ}$  were retained but the peak at  $7.3^{\circ}$  cannot be detected.

As the number of peaks present in the SC VAL is very low, it is hard to assign it to any specific known polymorph (244, 245). Despite being stored at a really high humidity for a prolonged period, the bulk crystallinity of the SC VAL has remained the same. Subtle changes can be observed, where the height of one of the humps changes slightly during the ageing time, but there have been no signs of crystalline peaks appearing, indicating retention of a bulk amorphous phase.

#### 6.4.1.2. Thermogravimetric Analysis (TGA)

The TGA thermograms for SC and am VAL stored at 85% RH are shown in Figure 6.2 and the mass losses observed in the thermograms for storage in 85% and 25% RH have been tabulated in Table 6.1. The mass loss trend has been the same for both forms of the VAL stored at 85% and 25% RH, and their mass loss trend matches that of the drug prior to ageing. The first mass loss region which takes place between 45 to 90 °C, can be attributed to the loss of moisture absorbed by the VAL while the other two mass losses attributed to the thermal decomposition of the VAL (247, 248, 264). There has not been any change in moisture uptake in SC VAL when stored at high humidity indicating low hygroscopicity as is expected in a semi-crystalline form whereas mass loss due to moisture uptake for Am VAL is significantly bigger than that of the semi-crystalline drug, indicating higher hygroscopicity of the amorphous form (256).

SC VAL demonstrated mass loss due to moisture of 0.43% in 85% RH which is only slightly higher than that of 25% RH, where the mass loss was 0.22%. However, for Am VAL, the mass loss induced by moisture uptake is 2.45% and 2.77% for systems stored at 85% and 25% RH, respectively. Despite having higher hygroscopicity than its semi-crystalline state, the moisture uptake for Am VAL was not higher when stored at high humidity as moisture uptake within the two systems are not much different from one another. The remaining two mass loss regions of Am VAL did not demonstrate any significant changes either, suggesting that the API in its amorphous form has not

undergone any moisture-induced degradation after being stored at high humidity for a period of 32 weeks.

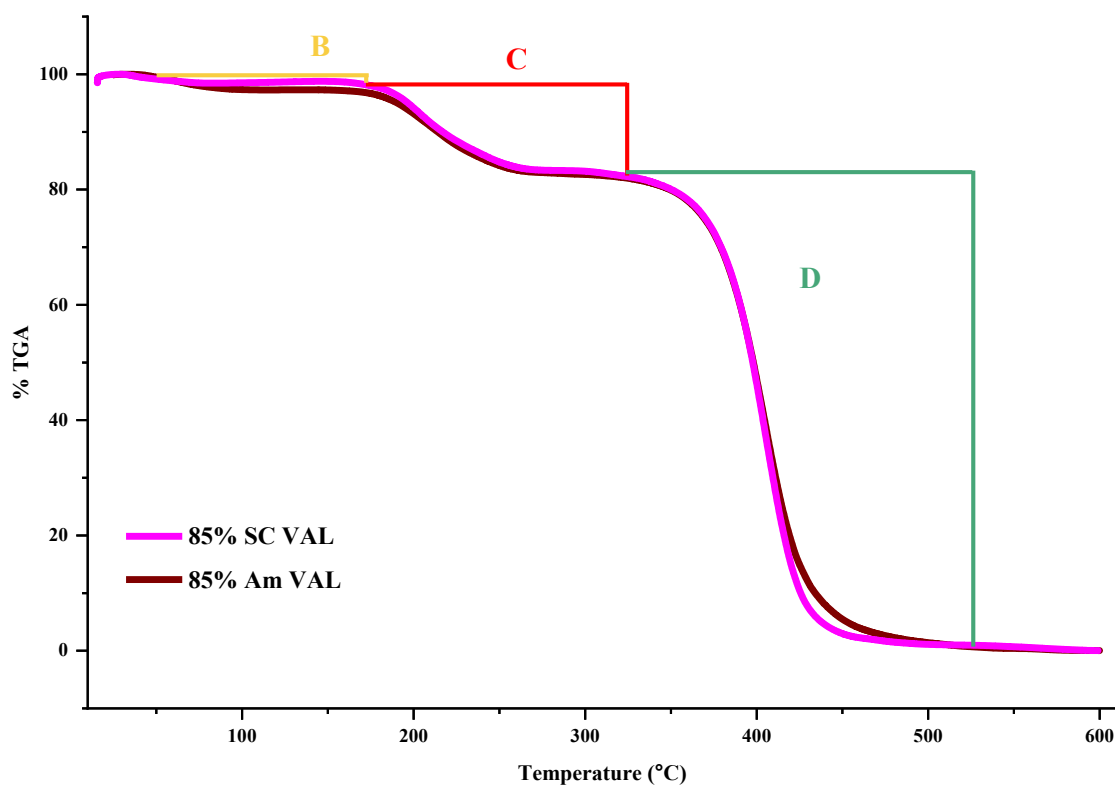


**Figure 6.2:** Thermogravimetric thermograms of SC VAL and Am VAL at week 32 for 85% RH.

**Table 6.1:** Mass loss trend in the thermogravimetric thermograms of SC VAL and Am VAL at week 32 for 85% and 25% RH.

| <u>Relative Humidity</u> | <u>VAL</u>       | <u>(EtOH mass loss – A) (%)</u> | <u>1<sup>st</sup> mass loss – B (%)</u> | <u>2<sup>nd</sup> mass loss – C (%)</u> | <u>3<sup>rd</sup> mass loss – D (%)</u> |
|--------------------------|------------------|---------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| <b><u>85% RH</u></b>     | Semi-crystalline | -                               | 0.43                                    | 13.28                                   | 71.44                                   |
|                          | Amorphous        | -                               | 2.45                                    | 13.31                                   | 75.06                                   |
| <b><u>25% RH</u></b>     | Semi-crystalline | -                               | 0.22                                    | 13.35                                   | 75.70                                   |
|                          | Amorphous        | -                               | 2.77                                    | 14.00                                   | 70.57                                   |

### 6.4.1.3. Temperature Modulated Differential Scanning Calorimetry (tmDSC)

To study the effect of adsorbed water in SC VAL and Am VAL stored at 85% and 25% RH as well as 45% RH (ambient room conditions), on the glass transition temperatures and melting point temperatures, tmDSC curves are measured, and only the first heating cycle has been considered. As reported in Chapter 4, moisture is removed during the first heating cycle and mixes the systems, making them amorphous, therefore a second heating cycle is not required here.

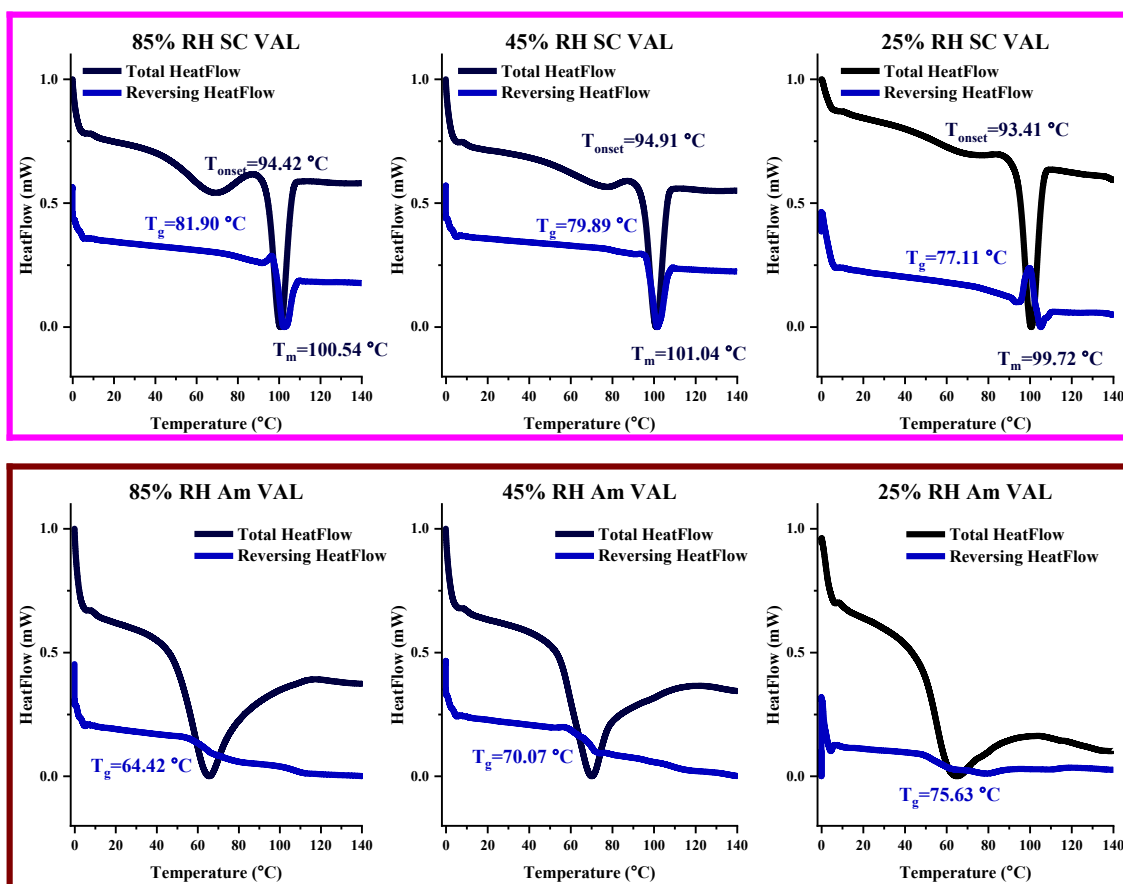


**Figure 6.3:** tmDSC plot with total and reversing heat flow signals of SC VAL (magenta) and Am VAL (maroon) at week 32 for 85%, 45% and 25% RH.

In most cases, water acts as a plasticiser, where the system becomes softer and flexible because the glass transition temperature ( $T_g$ ) gets lowered (149, 276). Figure 6.3 shows the total and reversing heatflow thermograms of SC VAL (bordered in magenta) and Am VAL (bordered in maroon) at 85%, 45% and 25% RH by means of tmDSC. Table 6.2 tabulates the  $T_g$  values of both SC and Am VALs stored at all three humidities. As humidity increases, the moisture adsorption increases slightly in SC VAL, with that the  $T_g$  values also keep increasing. At 25% RH, the  $T_g$  is 77.1 °C, whereas the value increases

to 79.9 °C and 81.9 °C for 45% and 85% RH, respectively. This system is semi-crystalline in all the cases as evidenced by the melting endotherm, commencing at 94.3±0.8 °C. However, there has not been a significant change in the  $T_g$  values of SC VAL, averaging at 79.6±2.4 °C across all the humidities, suggesting that the water does not have much effect in the semi-crystalline VAL.

**Table 6.2:** The  $T_g$ s after 32 weeks of storage in 85%, 45% and 25% RH, from SC VAL and Am VAL from Figure 6.3.

| <u>VAL</u>                  | <u><math>T_g</math> at 85% RH</u><br><u>(°C)</u> | <u><math>T_g</math> at 45% RH</u><br><u>(°C)</u> | <u><math>T_g</math> at 25% RH</u><br><u>(°C)</u> |
|-----------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| <b>Semi-crystalline VAL</b> | 81.90                                            | 79.89                                            | 77.11                                            |
| <b>Amorphous VAL</b>        | 64.42                                            | 70.07                                            | 75.63                                            |

However, in the case of Am VAL we observe the plasticising effect of water, as evidenced by the decrease in the  $T_g$  of the API. At 25% RH, the  $T_g$  is 75.63 °C, whereas the value decreases to 70.07 °C and 64.42 °C for 45% and 85% RH, respectively. This system is amorphous in all the cases as evidenced by the lack of melting endotherm at 101 °C. This is an interesting development when compared to SC VAL. Although the  $T_g$  values of SC VAL have increased slightly with increasing relative humidity, its average value has remained pretty much unchanged. This is possibly due to less adsorption of water by SC VAL than Am VAL, as demonstrated by the TGA data in section 6.4.1.2. Am VAL adsorbs more moisture under the same condition, thereby demonstrating a lowering of  $T_g$  due to the plasticising effect of adsorbed water.

#### 6.4.1.4. Polarised Light Microscopy (PLM)

Polarised light microscopy (PLM) gives us insight into the crystallinity of the systems. Crystallinity was observed in the form of optical birefringence within the solid amorphous particles.

Figure 6.4 shows the particles of SC VAL (bordered in magenta) and Am VAL (bordered in maroon) at weeks 0 and 32, for the systems stored at 85% RH. Figure 6.5 shows the increase in crystallinity (by area) in SC VAL and Am VAL after storing it 85% RH for 32 weeks.

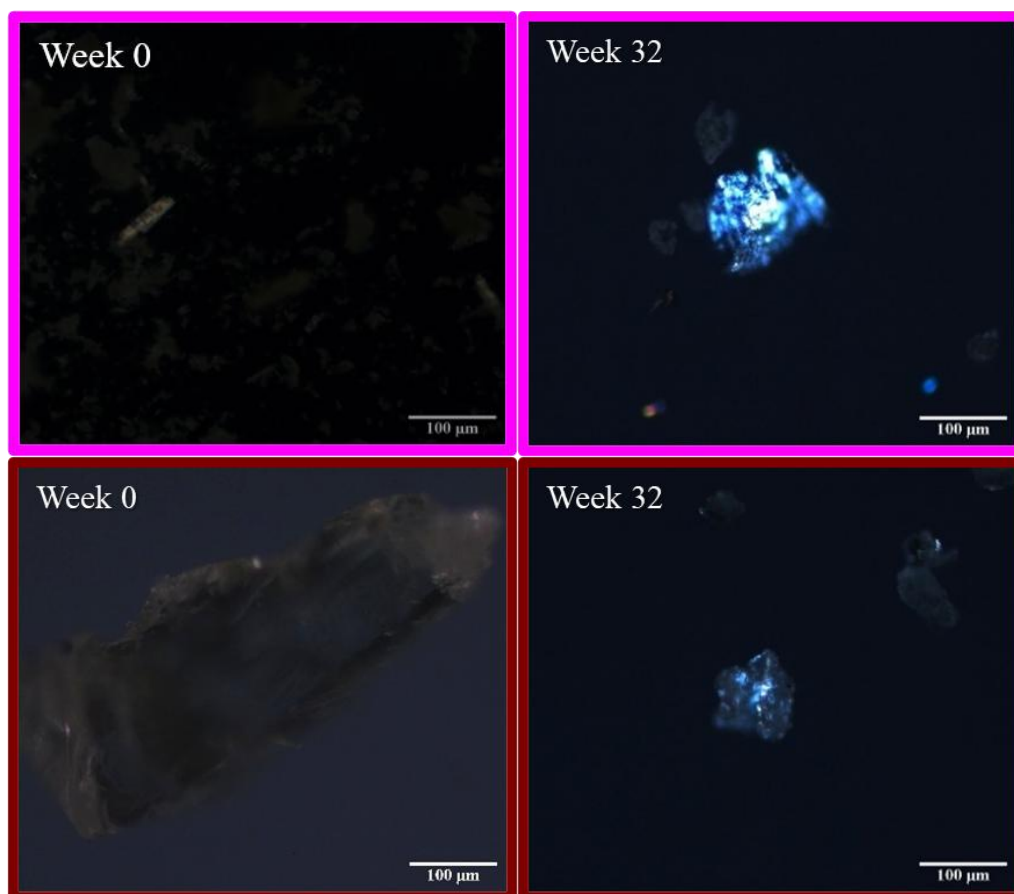


**Figure 6.4:** PLM images of SC VAL and Am VAL (bordered in magenta and maroon respectively) weeks 0 and 32 at 85% RH. Crystallinity was evaluated by analysing 10 images per sample, at a given time.

In the systems stored at 85% RH, crystallinity gradually increases, with the frequency of finding crystalline particles increasing over time. For SC VAL, the percentage of crystallinity increases from  $1.0 \pm 0.2\%$  to  $10.5\% \pm 1.3$  from week 0 to week 32. It can be said that a significant fraction of the system is still amorphous. This slight increase in crystallinity suggests that moisture in the surrounding facilitates in the nucleation of VAL molecules. Interestingly, no definite shape has been observed from the birefringence, which suggests that the crystallinity of SC VAL occurs in the form of small crystallites, aggregated together. Am VAL did not have any signs of crystallinity at week 0. However, after 32 weeks in 85% RH, crystallinity gradually increased, from 0% to  $1.7 \pm 0.8\%$ , indicating that the system remains essentially amorphous. At the lowest humidity of 25% RH, the images for both forms of VAL do not show any increase in crystallinity. PLM images of both SC and Am VAL stored at 25% RH for 32 weeks are presented in [Appendix C](#).

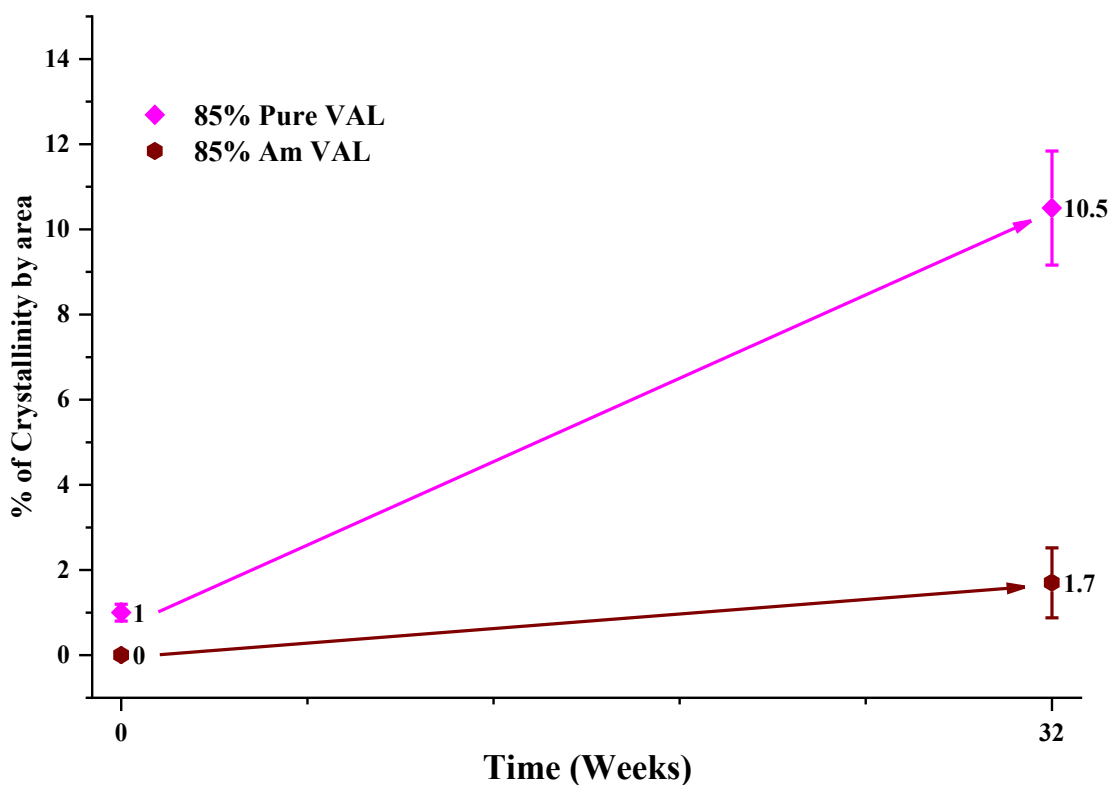


**Figure 6.5:** Increase in crystallinity in SC VAL and Am VAL at 85% RH over a period of 32 weeks, from PLM images. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size, with the error bars depicting the standard deviation.

With the increase in atmospheric moisture, SC VAL molecules at 85% RH may bond with water molecules thereby disrupting the VAL-VAL secondary bonds as the network of hydrogen bonds would allow water to diffuse through the system. This may enable migration of VAL molecules to form clusters of VAL which would establish stable crystal nuclei. This formation of a thermodynamically stable nucleation cluster happens over a relatively long period of time in SC VAL due to its semi-crystalline/semi-amorphous structure. Due to the lack of long-range order in VAL, as evidenced by its PDF pattern in [Chapter 5](#), its structure is similar to that of a metastable super-cooled liquid, which has a strong tendency to transform into a crystalline form. So far, no large crystals were observed in SC VAL after storage in 85% RH for 32 weeks, indicating that the crystallinity in VAL is dominated by nucleation and not growth or growth at a very slow rate on the existing nuclei present in the semi-crystalline system.

Am VAL adsorbs more moisture than SC VAL but does not recrystallise as much as the latter. This suggests that the semi-crystalline VAL recrystallises through heterogeneous nucleation, i.e., nucleation that occurs when existing crystals are present

in the system, which are absent in Am VAL. This increase in crystallinity from 0% to 1.7% in Am VAL may be due to homogeneous nucleation. However, it is not clear if the nucleation is homogeneous or heterogenous at the resolution of the PLM.

#### 6.4.1.5. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy has been employed on the SC VAL and Am VAL to study the influence of moisture on the drug when stored at different levels of humidity for a period of 32 weeks. Figure 6.6 shows the FT-IR spectra of both SC VAL and Am VAL stored in 85% RH after 32 weeks and the shifts in the vibrational peaks of the functional groups involved in hydrogen bonding for each of the systems are listed in Table 6.3. It is clear from the spectra that systems stored at 85% RH has absorbed significantly more water after 32 weeks, as is expected. FT-IR spectra for SC and Am VAL stored at 25% RH are presented and tabulated in Appendix C.

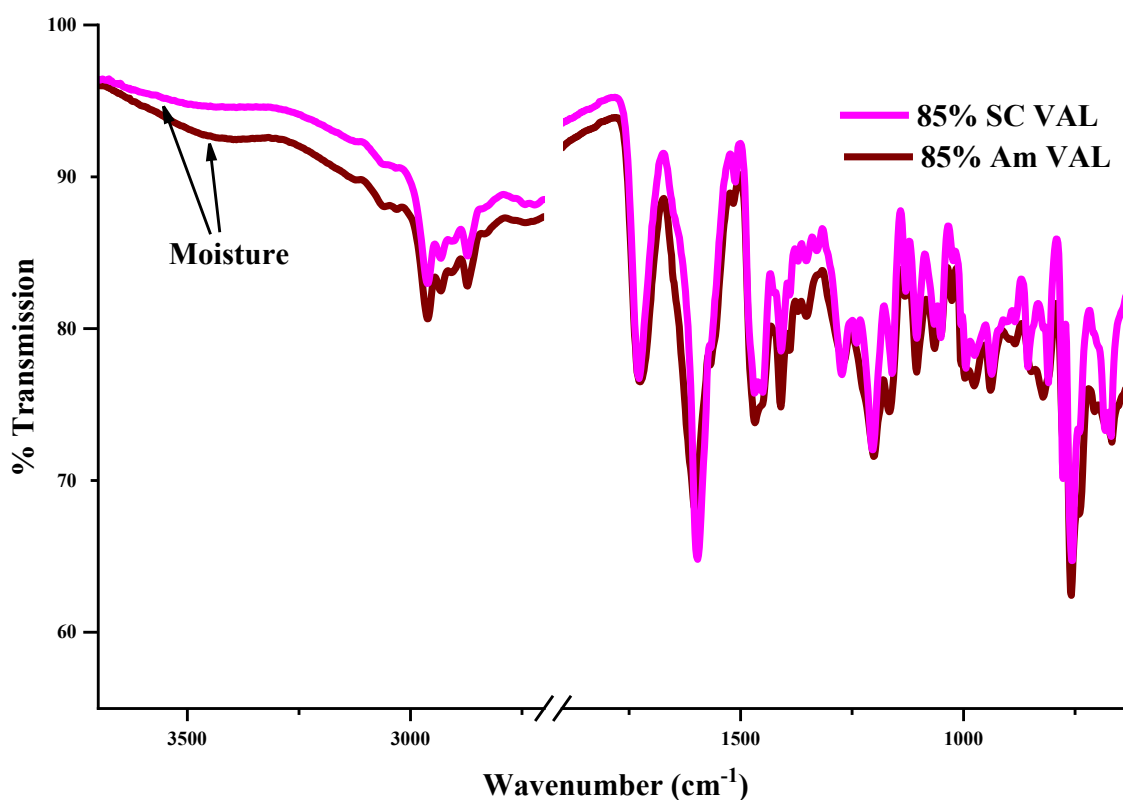


**Figure 6.6:** FT-IR spectra of semi-crystalline (SC) and amorphous (Am) VAL at 85% RH at week 32.

For SC VAL stored at 85% RH, the peak intensity of tetrazolyl H has increased and slightly shifted from  $3455\text{ cm}^{-1}$  to  $3452\text{ cm}^{-1}$ . This increase in peak intensity can be attributed to moisture as  $\text{H}_2\text{O}$  demonstrates a broad absorbance peak within the range of  $2700$  to  $3700\text{ cm}^{-1}$ , caused by the symmetric and asymmetric stretching of the OH bonds. No such change was observed in the systems stored at the lower humidities. The peak

position for O-H bonds of VAL has remained unshifted, at  $3116\text{ cm}^{-1}$  for both 85% and 25% RH humidities after 32 weeks of ageing. The stretching vibrational peaks for the acid C=O group and amide C=O group has remained pretty much unchanged for both the humidities, staying around  $1729\text{ cm}^{-1}$  and  $1598\text{ cm}^{-1}$ , respectively.

**Table 6.3:** Experimental Values of FT-IR spectra for SC VAL and Am VAL at 85% RH at week 0 and week 32 along with the shifts ( $\Delta$ ) in values of VAL prior to ageing (fresh).

| <b><u>Functional Groups</u></b>     | <b><u>Pure VAL</u></b> |                                      | <b><u>Am VAL</u></b> |                                       |
|-------------------------------------|------------------------|--------------------------------------|----------------------|---------------------------------------|
|                                     | <b><u>Week 0</u></b>   | <b><u>Week 32</u></b>                | <b><u>Week 0</u></b> | <b><u>Week 32</u></b>                 |
| (O-H) stretching                    | 3116                   | 3116                                 | 3116                 | 3122 ( $\Delta=+6$ )                  |
| $\nu(\text{NH})_{\text{tetrazole}}$ | 3455 (poor signal)     | 3452 (strong signal) ( $\Delta=-3$ ) | 3455 (poor signal)   | 3437 (strong signal) ( $\Delta=-18$ ) |
| Acid (C=O) stretching               | 1729                   | 1728 ( $\Delta=-1$ )                 | 1729                 | 1728 ( $\Delta=-1$ )                  |
| Amide (C=O) stretching              | 1597                   | 1597                                 | 1598                 | 1605 ( $\Delta=+7$ )                  |

For Am VAL stored at 85% RH, the peak intensity of tetrazolyl H has increased and slightly shift from  $3455\text{ cm}^{-1}$  to  $3437\text{ cm}^{-1}$  when stored at 85% RH, just like SC VAL. This change in peak intensity and position can be attributed to moisture as  $\text{H}_2\text{O}$  demonstrates a broad absorbance peak within the range of  $2700$  to  $3700\text{ cm}^{-1}$ , caused by the symmetric and asymmetric stretching of the O-H bonds. This peak intensity is higher for Am VAL, compared to SC VAL, consistent with the findings of TGA data presented in [Figure 6.6](#), where moisture adsorption is higher in the amorphous form of the VAL, indicating that the amorphous form of the drug is more hygroscopic than its semi-crystalline counterpart. No such change was observed by the system stored at 25% RH. The peak position for the O-H bond of Am VAL has changed slightly for both the humidities. It increased to  $3122\text{ cm}^{-1}$  from  $3116\text{ cm}^{-1}$  for systems stored at 85% RH but slightly decreased to  $3114\text{ cm}^{-1}$  when stored at 25% RH. The small increase in the peak shifts for 85% RH maybe influenced by the moisture adsorbed by the Am VAL but there is no increase in peak intensities for the O-H stretching frequency to suggest conclusively that this minor change is due to moisture. The stretching vibrational peaks for the acid C=O group for Am VAL has pretty much remained unchanged  $1728\text{ cm}^{-1}$  when stored at

85% RH but increased to  $1732\text{ cm}^{-1}$  for the system at 25% RH. The amide C=O group has increased to around  $1604\text{ cm}^{-1}$  for both the humidities. The lack of any significant shift in the carbonyl groups in amorphous VAL suggests that moisture does not interact with these groups.

At 25% RH, there appears to be a shoulder at  $1653\text{ cm}^{-1}$  for SC VAL and  $1651\text{ cm}^{-1}$  for Am VAL which can be assigned to the stretching vibrations of the aromatic rings of the API. However, for the system stored at 85% RH, that shoulder has gone undetected. This might be due to the presence of adsorbed water as water molecules demonstrate deformation around  $1660$  to  $1640\text{ cm}^{-1}$ .

The increase in the intensity of the tetrazolyl-H stretching peak at  $3452\text{ cm}^{-1}$  for SC VAL and  $3437\text{ cm}^{-1}$  for Am VAL and the undetected aromatic vibration shoulder at around  $1650\text{ cm}^{-1}$  is indicative of the presence of adsorbed moisture in the VALs stored at 85% RH, which could potentially influence secondary bonding interactions between VAL molecules in the systems. Furthermore, the peak intensity due to moisture is higher for Am VAL than SC VAL, indicating that amorphous form of the drug is more hygroscopic, consistent with the findings of thermogravimetric analysis in section 6.4.1.2.

#### 6.4.1.6. Raman Spectroscopy

Raman spectroscopy has been employed on the SC and Am VAL to study non-polar interactions within the system, something that FT-IR does not readily detect. Figure 6.7 and Table 6.4 demonstrate the changes in the Raman spectra of the SC and Am VAL to study the influence of moisture on the drug. Raman spectra for SC and Am VAL stored at 25% RH at week 32 are presented and tabulated in Appendix C.

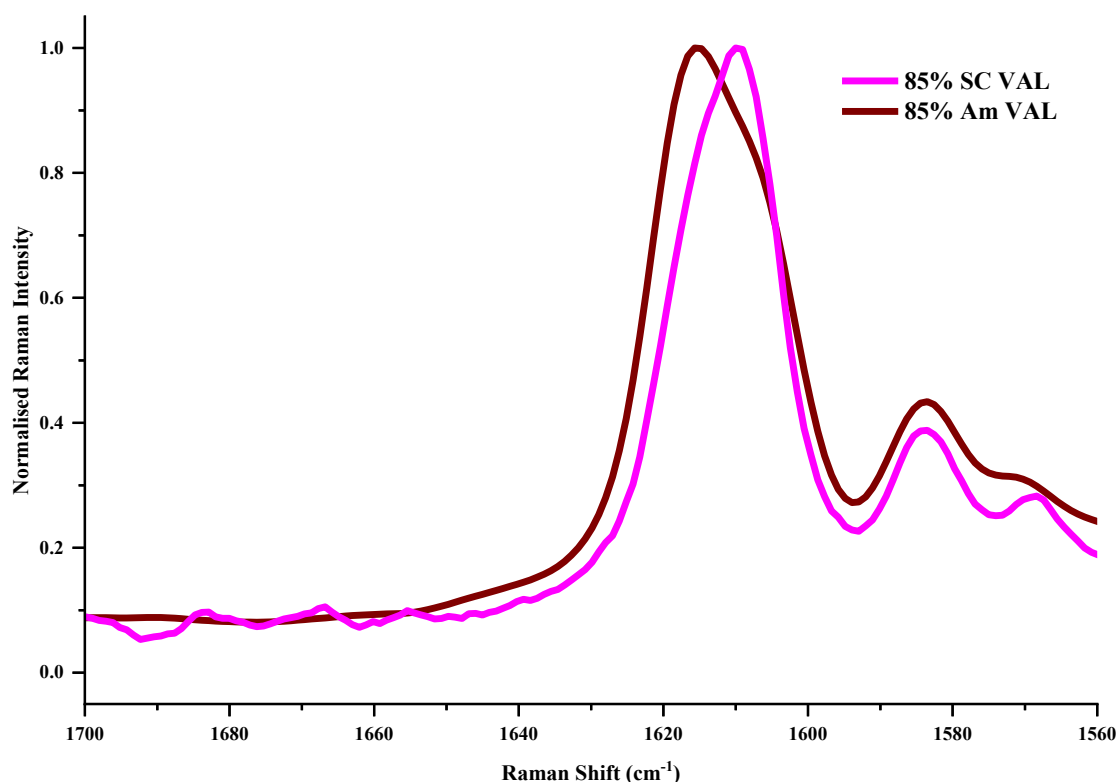


**Figure 6.7:** Raman spectra of semi-crystalline (SC) and amorphous (Am) VAL at 85% RH at week 32.

**Table 6.4:** Experimental Values of Raman spectra for SC VAL and Am VAL at 85% RH at week 32 along with the shifts ( $\Delta$ ) in values of VAL prior to ageing (fresh).

| <u>Functional Groups</u> | <u>Pure VAL</u> |                      | <u>Am VAL</u> |                      |
|--------------------------|-----------------|----------------------|---------------|----------------------|
|                          | <u>Week 0</u>   | <u>Week 32</u>       | <u>Week 0</u> | <u>Week 32</u>       |
| C=O (amide) <sub>s</sub> | 1608            | 1610 ( $\Delta=+2$ ) | 1609          | 1616 ( $\Delta=+7$ ) |
| N-H <sub>b</sub>         | 1564            | 1564                 | 1564          | 1564                 |
| C=C <sub>s</sub>         | 1583            | 1584 ( $\Delta=+1$ ) | 1583          | 1584 ( $\Delta=+1$ ) |

SC VAL shows two characteristic peaks at 1608 cm<sup>-1</sup> and 1583 cm<sup>-1</sup>, corresponding to C=O<sub>amide</sub> stretching and NH bending vibrations, respectively. The C=O<sub>amide</sub> increases slightly during the ageing process. It goes up to 1610 cm<sup>-1</sup> and 1609 cm<sup>-1</sup> for systems stored at 85% and 25% RH, respectively. The NH peaks pretty much remain unshifted for the system stored at all humidities. Other than the very minor

changes in the peak position, it is hard to observe any significant differences between all three systems brought about by moisture.

Am VAL shows two characteristic peaks at  $1609\text{ cm}^{-1}$  and  $1583\text{ cm}^{-1}$ , corresponding to  $\text{C}=\text{O}_{\text{amide}}$  stretching and NH bending vibrations, respectively. The  $\text{C}=\text{O}_{\text{amide}}$  increases during the ageing process. It goes up to  $1616\text{ cm}^{-1}$  and  $1615\text{ cm}^{-1}$  for systems stored at 85% and 25% RH. The NH peaks have not undergone any change in peak positions for both forms of VAL in both the relative humidities. The C=C stretching vibrations have increased by  $1\text{ cm}^{-1}$  for both SC and Am VAL to  $1584\text{ cm}^{-1}$ , when stored at 85% RH, possibly indicating a disruption in the  $\pi$ - $\pi$  stacking interaction of the self-assembled VAL molecules, possibly due to interaction with water molecules, as the water molecules are small enough to get between the stacked aromatic rings, as no such change was observed in both the VAL forms stored at 25% RH (135).

This possible increase in shift or blue shift of the C=O bond in Am VAL could be indicative of the formation of hydrogen bonding interaction with water, where the oxygen of the carbonyl group is bonding with the H of water, increasing the electron density within the C=O bond and thereby strengthening the bond, which reduces the bond length. The shift is the least for Am VAL stored at 25% RH, consistent with the hydrogen bonding being more abundant in 85% RH. It is important to note that the spectral base line for the system stored at 25% RH is higher than those of the system stored at 85% RH, suggesting that an increase in moisture decreases the background as variable moisture content introduces spectral defects due the fluorescence quenching effect of moisture on pharmaceutical compounds (277, 278).

Other than the changes in the peak positions of the amide and C=C groups of the SC and Am VAL, that suggest the formation of hydrogen bonding interaction with water and the differences in the spectral baseline of the system stored at 25%, no other moisture-induced differences could be observed in the systems when stored at 85% RH.

#### 6.4.1.7. Summary of Case Study I

The pure drug VAL, in both its semi-crystalline and amorphous forms, does not significantly recrystallise in the presence of moisture. When it does recrystallise, it does so in the presence of pre-existing crystal nuclei, aided by the presence of high moisture content, where the secondary bonds between the VAL molecules are disrupted by water molecules, enabling the growth of stable crystal nuclei. This presence of pre-existing

crystals which aids the formation of VAL crystals suggests that recrystallisation in VAL is occurring by heterogeneous nucleation.

#### **6.4.2. Case study II: Co-amorphous systems of VAL and NIC**

Melt amorphised CAS was prepared by grinding an equimolar mixture of SC VAL and NIC, followed by melting it at a temperature higher than both the components' melting points, and then rapidly air-cooling to room temperature. It transformed into a glassy and transparent melted state which returns to being white upon extraction from the processing vial. At the lowest humidity of 25%, it has maintained its loose powdery state in the total 32-week storage time period. The CAS at 85% RH was no longer powdery after 4 weeks of storage, but had transformed into a glassy clump, that would adhere to the walls of the glass bottle. It breaks into its powdery state when enough pressure is applied, as it would in a mortar and pestle.

Ball milled CAS was prepared by milling equimolar amounts of VAL and NIC in a mixer mill with two stainless steel balls at 30 Hz for 4 hours. The mixture of the two components transformed from white powder to a finely milled powder with a yellowish tinge. At the lowest humidity of 25%, it has maintained its loose powdery state in the total 32-week duration. The CAS at 85% RH was no longer powdery but had transformed into a rock-like solid and the colour of the aged system was bright orange after 4 weeks of storage. It would break into its powdery state when enough pressure was applied.

Spray dried CAS was prepared by dissolving an equimolar mixture of VAL and NIC in a solvent mixture of equal parts of absolute ethanol and water, which was atomised into tiny droplets by a two-fluid nozzle and rapidly dried with hot gas. The sample was collected from the collection jar as well as the inner wall of the cyclone, where the powder adhered to. The powder extracted is white in colour, loose and appeared very fine. At the low humidity of 25% RH, it has maintained its loose powdery state in the total 32-week time period. However, the CAS at 85% RH was no longer powdery after 4 weeks of storage, but had transformed into a glassy clump, that would adhere to the walls of the glass bottle. It would break into its powdery state readily under pressure.

Slurry mixed CAS was prepared by dissolving an equimolar mixture of VAL and NIC in boiling ethanol, then subjecting it to vacuum and moderately high temperature in a rotary evaporator to obtain an amorphous powder. It transformed into a glassy and transparent melted state which appeared foamy during the extraction process. Even with

the complete removal of solvent, the powder dried in that “foamy” state, which crumbles into fine white powder on stress. At the low humidities of 25% RH, it has maintained its loose powdery state in the total 32-week duration. However, the CAS at 85% RH was no longer powdery, but had transformed into a glassy, sticky clump, that would adhere to the walls of the glass bottle after 4 weeks of storage. It lost its glue-like tendencies when ground in a mortar and pestle and transforms into a powdery state.

#### 6.4.2.1. Powder X-Ray Diffraction (pXRD)

Figure 6.8 (a to d) shows that MA, SD, and SL CASs stored in 25% RH do not form any crystalline peaks in the continued ageing period of 32 weeks. At the lower humidities, a low water content is unlikely to have sufficiently disrupted the hydrogen bonds between the amorphous NIC and VAL molecules, thereby maintaining the amorphous state of the CASs. However, pXRD was able to detect a small peak at  $14.8^\circ$  for BM CAS, indicating a minor crystalline phase. This would suggest that the binary system can be prone to recrystallisation even at low relative humidity at ambient conditions, if enough time has passed from its time of preparation i.e., recrystallisation due to kinetics. This crystallinity could also be due to the fact that BM CAS already had NIC crystals detected by PLM in Chapter 5 and these have undergone growth in the 32-week period. Nevertheless, it is safe to conclude that all four CASs have retained their bulk amorphisity at the low relative humidity of 25% for 32 weeks.

The physical differences between the different CASs stored at different relative humidity can be analysed at a structural level. Figure 6.8 and Figure 6.9 demonstrates the pXRD patterns of MA, BM, SD, and SL CASs for 25% and 85% RH respectively over a period of 32 weeks. Figure 6.9 (a) and (c) demonstrates the appearance of crystalline peaks in the X-ray pattern of MA and SD CASs after 4 weeks of storage in 85% RH. The crystalline peaks index to that of the excipient NIC. By week 32, the recrystallisation of NIC is complete, with all the highly intense crystalline peak of NIC detectable in the two pXRD patterns. Interestingly, no peaks of VAL can be detected in the X-ray pattern, indicating the VAL has stayed amorphous.

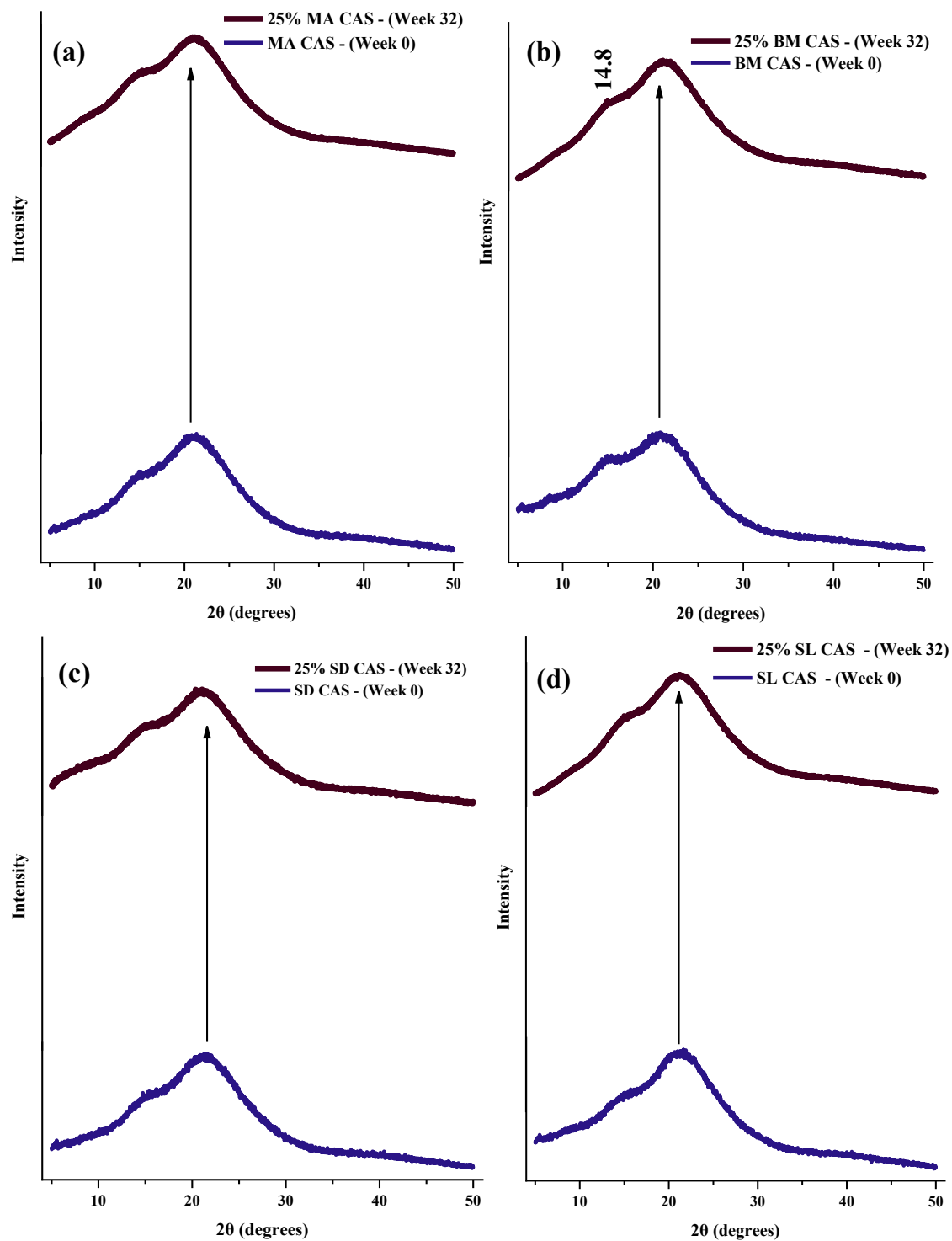


**Figure 6.8:** PXRD patterns of CASs stored at 25% RH for 32 weeks. (a) MA CAS (b) BM CAS (c) SD CAS and (d) SL CAS.

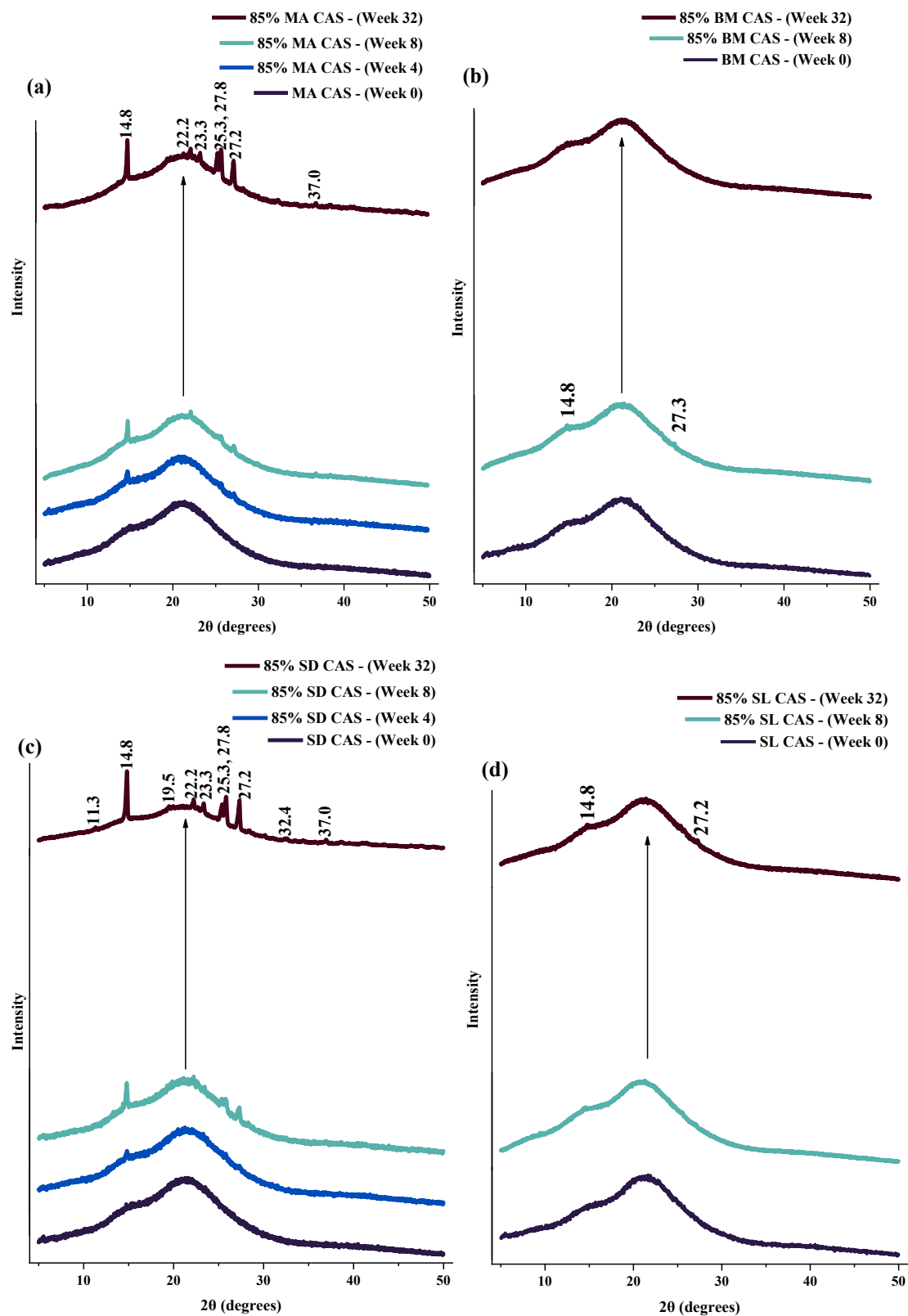


**Figure 6.9:** PXRD patterns of CASs stored at 85% RH for 32 weeks, with the peaks reflecting that of NIC. (a) MA CAS (b) BM CAS (c) SD CAS and (d) SL CAS.

Figure 6.9 (b) demonstrates pXRD patterns of BM CAS stored at 85% RH. Crystalline peaks in the pXRD appear at  $14.8^\circ$  and  $27.3^\circ$  after 4 weeks of storage in 85%

RH, both of which can be indexed to NIC. However, as the time went on, the peaks often go undetected. This is quite intriguing as this has not been observed in any other CASs. This “disappearance” of NIC peaks over the course of 32 weeks could be attributed to the inhomogeneous nature of the ball milled CAS. What could be happening is that the recrystallisation of NIC in the BM CAS occurs erratically through the binary system, wherever the amount of pre-existing NIC crystallites is the highest. The presence of water molecules in the atmosphere would disrupt the hydrogen bonding interaction between VAL and NIC, facilitating the migration of NIC through the mixture to grow the clusters of NIC crystallites, causing an excess of NIC in certain areas of the mixture. This breakdown of the single-phase homogeneity of the CAS under moisture impacts the bulk characteristics of the system under analysis. This means that the sample taken from the jar for X-ray analysis may not possess enough NIC crystals to generate peaks in the data. However, evidence of crystallinity in the BM system when stored at 85% RH can be found in PLM and by thermal analysis, as explained in later sections.

Figure 6.9 (d) demonstrates pXRD patterns of SL CAS stored at 85% RH. After 32 weeks of storage in 85% RH, small peaks appear at  $14.8^\circ$  and  $27.2^\circ$ , which can be indexed to that of NIC, indicating that the drug VAL has not recrystallised.

All four CASs have shown signs of NIC recrystallisation stored in the 85% RH by pXRD but at different time points. MA and SD CASs showed evidence of NIC crystallinity after 4 weeks, BM CAS demonstrated NIC crystallinity after 8 weeks in the humidity chamber. SL CAS was the most stable against NIC recrystallisation as it showed two small peaks after 32 weeks in the ageing chamber. At 25% RH, all four CASs have stayed amorphous when examined by pXRD for 32 weeks, with BM CAS showing minor crystallinity, thereby suggesting that the metastable amorphous system can recrystallise slowly over long periods even in the absence of excess moisture.

#### 6.4.2.2. Thermogravimetric Analysis (TGA)

The TGA thermograms for MA, BM, SD, and SL CASs stored at 85% RH are shown in Figure 6.10 and the mass losses observed in the thermograms for storage in 85% and 25% RH have been tabulated in Table 6.5. The loss observed in MA and BM CASs follows the same trend as that of VAL in section 6.4.1.2. The TGA thermogram of MA and BM CASs has three mass loss regions just like VAL. In SD and SL CASs, presence of ethanol has been detected in Chapter 5 in the first mass loss region. The rest of the mass loss regions are similar to that of the MA and the BM CASs for the 32-week

duration. However, the moisture uptake of CASs stored at 2 different humidities have been similar to that of Am VAL for the 32-week time period.

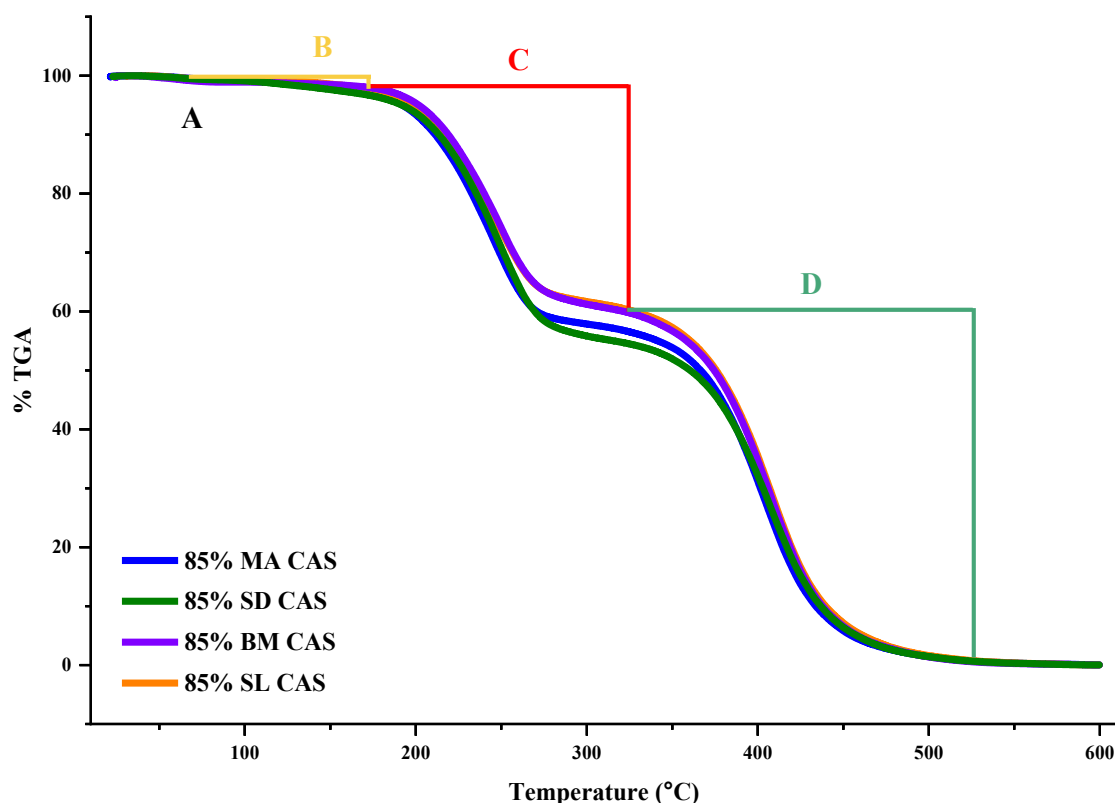


**Figure 6.10:** Thermogravimetric thermograms of MA, BM, SD, and SL CAS at week 32 for 85% RH.

At 25% RH, the moisture uptake is really low at 0.18%, 0.15%, 0.17 and 0.45% for the MA, BM, SD, and SL CASs, respectively. With increasing moisture content surrounding the CAS, the moisture uptake increases. When stored at 85% RH, the mass losses due to moisture are 1.52%, 1.18%, 1.57%, and 0.87% for the MA, BM, SD, and SL CASs respectively, same as Am VAL. This rise in moisture absorption indicates that the CAS is hygroscopic and storage at higher humidity leads to increased moisture uptake which would ultimately lead to VAL-NIC bond disruption. The second and the third mass loss regions in all four CASs are approximately  $36.1 \pm 1.1\%$  and  $53.9 \pm 1.4\%$  for 85% RH and  $34.6 \pm 0.3\%$  and  $57.1 \pm 0.2\%$  for 25% RH, respectively. The increase adsorption of moisture in the systems aids in the recrystallisation of the system, evidenced by pXRD findings in [Figure 6.8](#) and [Figure 6.9](#), where systems stored at 25% RH retained their amorphous state whilst those stored at 85% RH did not. Given that the mass loss for all four systems at two different humidities do not have any major chemical changes, it suggests that no moisture induced chemical degradation has undergone in the CASs.

**Table 6.5:** Mass loss trend in the thermogravimetric thermograms of MA, BM, SD, and SL CAS at week 32 for 85% and 25% RH.

| <b><u>Relative Humidity</u></b> | <b><u>Co-amorphous systems</u></b> | <b><u>(EtOH mass loss – A) (%)</u></b> | <b><u>1<sup>st</sup> mass loss – B (%)</u></b> | <b><u>2<sup>nd</sup> mass loss – C (%)</u></b> | <b><u>3<sup>rd</sup> mass loss – D (%)</u></b> |
|---------------------------------|------------------------------------|----------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|
| <b><u>85%</u></b>               | Melt Amorphisation                 | -                                      | 1.52                                           | 36.61                                          | 52.04                                          |
|                                 | Ball Milling                       | -                                      | 1.18                                           | 34.53                                          | 55.77                                          |
|                                 | Spray Drying                       | 0.53                                   | 1.57                                           | 39.01                                          | 50.81                                          |
|                                 | Slurry Mixing                      | 0.58                                   | 0.87                                           | 34.17                                          | 56.81                                          |
| <b><u>25%</u></b>               | Melt Amorphisation                 | -                                      | 0.18                                           | 35.35                                          | 56.73                                          |
|                                 | Ball Milling                       | -                                      | 0.15                                           | 34.32                                          | 57.75                                          |
|                                 | Spray Drying                       | 0.33                                   | 0.17                                           | 34.65                                          | 56.94                                          |
|                                 | Slurry Mixing                      | 0.16                                   | 0.45                                           | 33.98                                          | 57.08                                          |

The subtle differences between the systems could potentially be due to disruption in the uniformity of the systems, caused by moisture-induced phase separation and recrystallisation of NIC.

#### 6.4.2.3. Temperature Modulated Differential Scanning Calorimetry (tmDSC)

To study the effect of adsorbed water in CASs stored at 85% and 25% RH, on the glass transition temperatures and melting point temperatures, tmDSC curves are measured, and only the first heating cycle has been considered. As reported in [Chapter 4](#), moisture is removed during the first heating cycle and mixes the systems, making them amorphous, therefore a second heating cycle is not required here.

The occurrence of phase separation has been detected by tmDSC for all the CASs at both 85% RH and 25% RH. The trend in phase separation in the CASs stored at 25% RH has been shown in [Figure 6.11](#) and laid out in [Table 6.6](#) and that of at 85% RH is presented in [Figure 6.13](#) and [Table 6.7](#). The tmDSC data reveals more information on the crystallinity of the system that was not detected in the pXRD data presented in [Figure 6.8](#)

(a to d), where all the systems were essentially amorphous. tmDSC thermograms for CASs stored at 25% RH after 32 weeks are presented in [Appendix C](#).

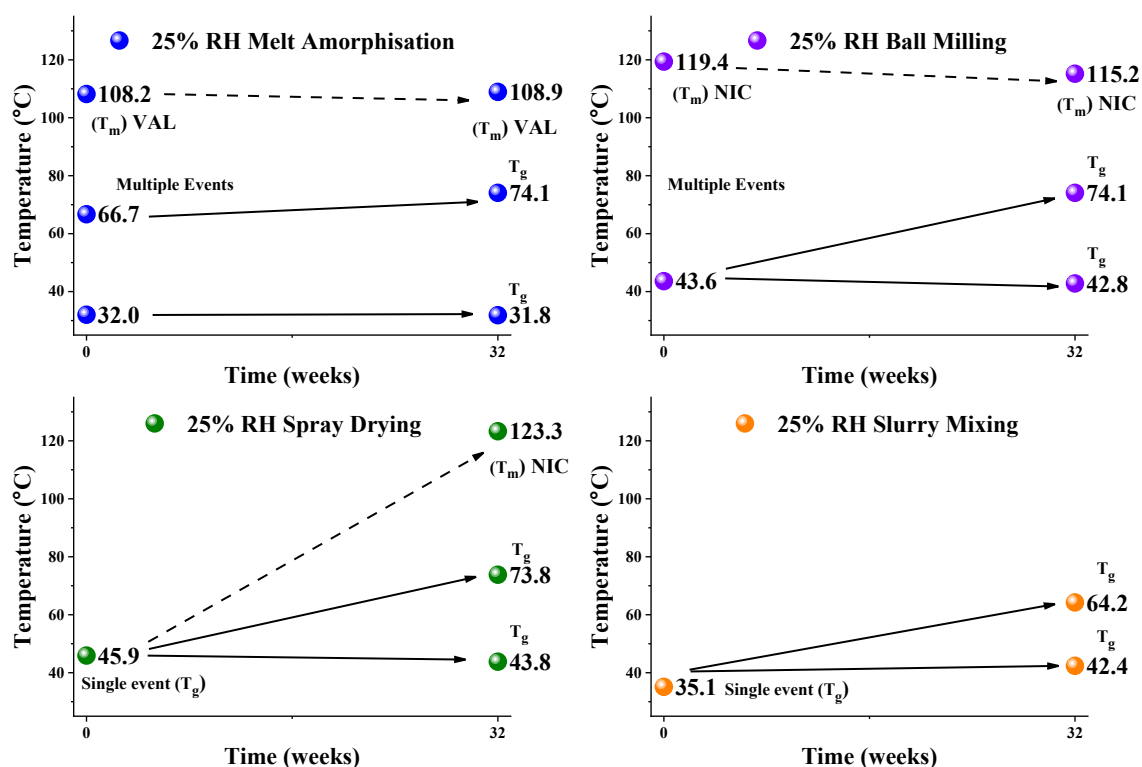


**Figure 6.11:** The occurrence of multiple endothermic events after 32 weeks of storage in 25% RH, from the freshly prepared CASs.

Starting with the MA CAS, at 25% RH in 32 weeks of storage, three endothermic events have been observed, namely two glass transition events at 31.8 °C and 74.1 °C and a melting point event at 108.9 °C, and these three events were already observed in the freshly prepared sample, as stated in [Chapter 5](#) and [Appendix B](#). The melting endotherm reflects the melting point of VAL, which is 103.5 °C, as was observed in the first heating cycle. It appears that the recrystallised drug, evidenced by the presence of a melting endothermic peak has gone undetected by pXRD. Prior to ageing, the system had two glass transition and one melting endotherms, indicating the occurrence of amorphous-amorphous phase separation (AAPS) and recrystallisation in the X-ray amorphous system. The  $T_g$  of 32 °C has barely shifted, which would suggest that VAL and NIC are still bonded to each other. However, the increase of  $T_g$  from initial amorphous phase from 66.7 °C in the freshly prepared sample to 74.1 °C, a value close to the  $T_g$  of VAL, after 32 weeks in 25% RH indicates that some VAL molecules may have overcome the hydrogen bonding and is now existing freely in the CAS, suggesting that the system has phase separated after 32 weeks of storage, even at low humidity.

**Table 6.6:** The occurrence of multiple endothermic events after 32 weeks of storage in 25% RH, from the freshly prepared CASs, tabulated from **Figure 6.11**.

| <u>Co-<br/>amorphous<br/>System</u> | <u>Endothermic event at Week 0</u><br><u>(°C)</u> |                                      | <u>Endothermic event(s) at Week</u><br><u>32 (°C)</u> |                      |
|-------------------------------------|---------------------------------------------------|--------------------------------------|-------------------------------------------------------|----------------------|
|                                     | <b>T<sub>g</sub> (First Heating)</b>              | <b>T<sub>m</sub> (First Heating)</b> | <b>T<sub>g</sub></b>                                  | <b>T<sub>m</sub></b> |
| Melt Amorphisation                  | 32.0, 66.7                                        | 108.2 (VAL)                          | 31.8, 74.1                                            | 108.9 (VAL)          |
| Ball Milling                        | 43.6                                              | 119.4 (NIC)                          | 42.8, 74.1                                            | 115.2 (NIC)          |
| Spray Drying                        | 45.9                                              | -                                    | 43.8, 73.8                                            | 123.3 (NIC)          |
| Slurry Mixing                       | 35.1                                              | -                                    | 42.4, 64.2                                            | -                    |

For the BM CAS stored at 25% RH, three endothermic events have been observed, namely two glass transition events, with  $T_g$  at 42.8 °C and 74.1 °C and a melting point event at 115.2 °C, during the 32 weeks of ageing. The  $T_m$  reflects the melting point of NIC, which is 129.7 °C and along with the first  $T_g$ , these two events were already observed in the freshly prepared sample, as stated in [Chapter 5](#) and [Appendix B](#), where signs of NIC recrystallisation had been evidenced by PLM and tmDSC, indicating its inhomogeneity. The first  $T_g$  is observed at 42.8 °C, which is only a bit lower than the original  $T_g$  of the BM CAS, which was at 43.6 °C (50.1 °C is the  $T_g$  obtained from second heating). This lowering of  $T_g$  could be due to the presence of more excess NIC, unbonded to VAL in the sample, which lowers the overall  $T_g$  of the system. The occurrence of the second  $T_g$  at 74.1 °C, is indicative of AAPS, as this value is close to the value of VAL, as was observed in the MA CAS stored at 25% RH for 32 weeks, where VAL is existing freely. The presence of NIC crystallites along with phase separated VAL could be a result of inhomogeneity, and it is getting detected by tmDSC and pXRD in [Figure 6.8 \(b\)](#). This excess of NIC, whether crystalline or amorphous is indicative of the lack of uniform mixing in the BM CAS, which plays a role in advancing phase separation of binary system in the absence of moisture.

In the SD CAS, three endothermic events have been observed when stored at 25% RH, namely  $T_g$  at 43.8 °C and 73.8 °C and  $T_m$  at 123.3 °C. The melting endotherm can be assigned to NIC. SD CAS was a homogeneous system but has now undergone phase

separation during the 32-week period it was stored at low humidity. This suggests that small-scale recrystallisation is occurring even when the moisture content in the surrounding is low if enough time has passed. The occurrence of two glass transition events, with the second value similar to those found in MA and BM CASs, indicating the presence of unbonded VAL freely existing in the CAS. The slightly lowering of  $T_g$  from 45.9 °C to 43.8 °C, can be due to the occurrence of amorphous, hydrated NIC that is unbonded to VAL, which drives the overall  $T_g$  of the CAS to a lower value. The formation of two glass transition events and a melting endotherm indicate the occurrence of AAPS and recrystallisation when stored at low humidity for 32 weeks.

For the SL CAS, although prepared by a solvent evaporation technique, it has not behaved in the same manner as the SD CAS when stored at 25% RH for 32 weeks. Two glass transition events have been observed at 42.4 °C and 64.2 °C after ageing. The  $T_g$  value at 42.4 °C has increased by 7.3 °C from the  $T_g$  value of 35.1 °C in the first heating cycle (prior to ageing). This increase in  $T_g$  could be due to gradual dehydration of the system when stored at a dry environment (caused by desiccants), and this eliminates the plasticising effect of water, leading to an increase in the glass transition. The second  $T_g$  at 64.2 °C indicates AAPS, and this event is occurring due to an amorphous phase where the VAL content is higher as NIC is crystallising out, as systems with higher VAL content have  $T_g$  values closer to that of the API, as seen in [Chapter 4](#). Although recrystallisation has not been observed in SL CAS, this system still undergoes AAPS at relatively low humidity, suggesting that phase separation occurs even in the absence of moisture.

tmDSC thermograms for the MA, BM, SD and SL CASs (bordered in blue, purple, green and orange respectively) stored at 85% RH for 32 weeks are presented [Figure 6.12](#) with both the total and reversing heatflow thermograms, and the data has been presented in [Figure 6.13](#) and tabulated in [Table 6.7](#). As the humidity increases, the phase separation becomes more apparent.

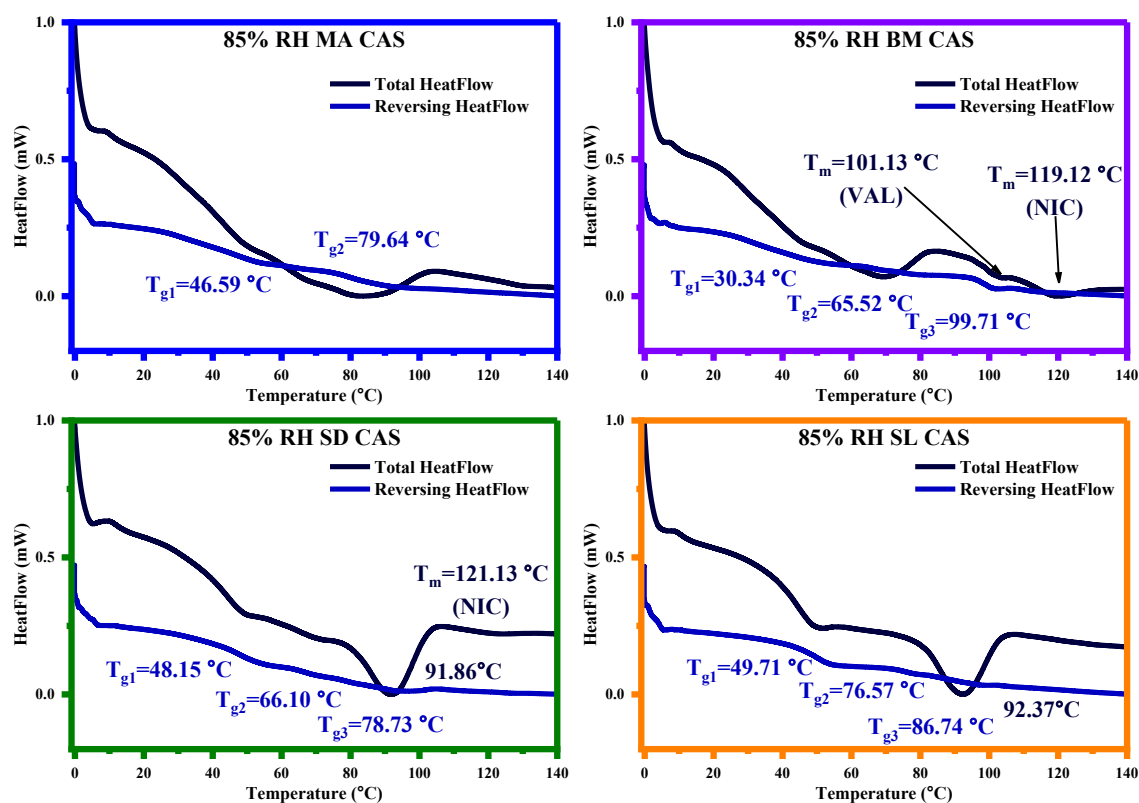


**Figure 6.12:** tmDSC plot with total and reversing heat flow signals of MA, BM, SL and SD CASs at week 32 for 85% RH.

The MA CAS had three endothermic events, prior to ageing. After 32 weeks of storage in 85% RH, two glass transition events (observed in the reversing heatflow thermogram) are at 46.6 °C and 79.6 °C, have increased by 15 °C and 13 °C respectively, from the  $T_g$  values of the freshly prepared sample. With occurrence of NIC recrystallisation, as evidenced by pXRD data in section 6.4.2.1, there is a possibility that there is an excess of VAL in the CAS which has altered the composition, thereby, increasing the overall glass transition of the system, i.e. the increase in the first  $T_g$ . Furthermore, the increase in the second  $T_g$  at 79.6 °C, which was already present in the freshly prepared sample, is due to the presence of some amorphous VAL molecules bonded to water, existing outside the CAS, due to the NIC crystallising out. There appears to be no melting point endotherm in the system, indicating that system does not have any signs of crystallinity, which is contrary to the pXRD patterns as seen in Figure 6.9 (a), where NIC crystallinity can be detected very strongly. Additionally, the apparent disappearance of the melting endotherm of crystalline VAL, that was present in the freshly prepared sample, suggests that the occurrence of crystallinity is random or that VAL has melted and mixed changing the composition, leading to AAPS, indicating that inhomogeneity is present in the MA CAS, before and after the ageing. Secondly, the lack

of melting endotherm would suggest that the system only has amorphous forms of VAL and NIC present in it. However, taking into account the pXRD patterns where crystalline peaks of this MA CAS strongly reflect that of NIC suggests that NIC has recrystallised but the tmDSC does not detect its presence.

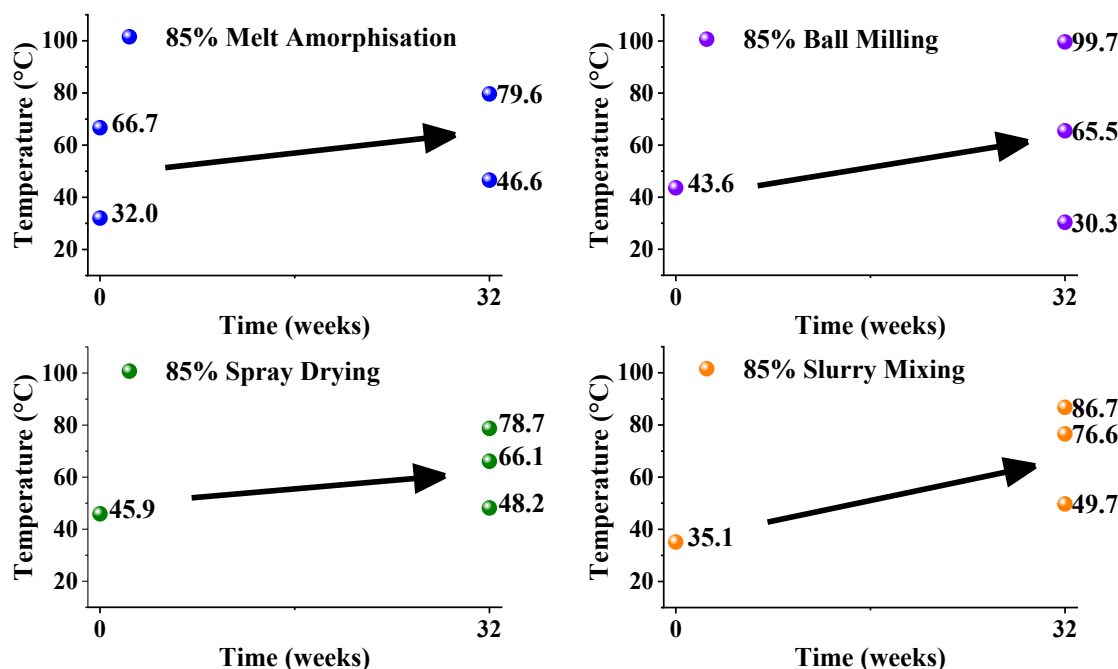


**Figure 6.13:** The occurrence of multiple  $T_g$ s after 32 weeks of storage in 85% RH, from the freshly prepared CASs.

The tmDSC data for the BM CAS system stored at 85% RH for 32 weeks has also exhibited phase separation. Three glass transition events have been observed in the reversing heatflow thermogram at 30.3 °C, 65.5 °C and 99.7 °C. The first  $T_g$  has reduced by 13 °C from the value of the freshly prepared sample. This lowering of  $T_g$  can be attributed to the presence of excess amorphous, hydrated NIC content in the sample, which shifts the  $T_g$  value towards that of pure NIC, which is -7.3 °C, as NIC is a hydrotrope and it can easily bond with water molecules and is more susceptible to the plasticising effect of water (149, 251). The second  $T_g$  at 66.5 °C is close to the  $T_g$  value of Am VAL stored at 85% RH, suggesting the presence of Am VAL freely existing in the BM CAS. The third  $T_g$  at 99.7 °C could be due to the presence of moisture, likely forming hydrate, which could explain the excessive increase in the glass transition temperature. The total heatflow thermogram supports the presence of recrystallised NIC and VAL by demonstrating melting endotherms at 119.1 °C and 101.1 °C respectively. The presence of a melting endotherm of VAL suggests that the  $T_g$  at 99.7 °C could be a hydrate formed with crystalline VAL. The system not only has three amorphous phases but potentially

two crystalline phases, indicating that the system has undergone phase separation and recrystallisation and is inhomogeneous.

**Table 6.7:** The occurrence of multiple  $T_g$ s and  $T_m$ s after 32 weeks of storage in 85% RH, from the freshly prepared, tabulated from **Figure 6.13**.

| <b><u>Co-amorphous System</u></b> | <b><u><math>T_g</math> at Week 0 (°C)</u></b> | <b><u><math>T_g</math> (s) at Week 32 (°C)</u></b> | <b><u><math>T_m</math> (s) at Week 32 (°C)</u></b> |
|-----------------------------------|-----------------------------------------------|----------------------------------------------------|----------------------------------------------------|
| Melt Amorphisation                | 66.7, 32.0                                    | 79.6, 46.6                                         | -                                                  |
| Ball Milling                      | 43.6                                          | 99.7, 65.5, 30.3                                   | 101.1 (VAL),<br>119.1 (NIC)                        |
| Spray Drying                      | 45.9                                          | 78.7, 66.1, 48.2                                   | 121.1 (NIC)                                        |
| Slurry Mixing                     | 35.1                                          | 86.7, 76.6, 49.7                                   | -                                                  |

The SD CAS system stored at 85% RH for 32 weeks has exhibited phase separation, just like the MA and BM CASs. Three glass transition events have been observed in the reversing heatflow thermogram at 48.2 °C, 66.1 °C and 78.7 °C after ageing. The total heatflow thermogram shows two endothermic events, one big peak at around 91.9 °C, which indicative of adsorbed moisture and a small peak at 121.1 °C, which reflects the melting point of NIC. The presence of the melting point peak of NIC suggests that an excess of NIC is existing outside the binary system, indicating a disruption of hydrogen bonding between the API and the excipient, causing a phase separation. The first  $T_g$  is close to the value of the freshly prepared sample (45.9 °C), indicates that the system is partially stable. The partial stability is due to the occurrence of two additional glass transition events thereby indicating that the system is not entirely homogeneous anymore. The second  $T_g$  at 66.1 °C is close to the  $T_g$  value of Am VAL stored at 85% RH, suggesting the presence of Am VAL freely existing in the SD CAS. The third  $T_g$  at 78.7 °C is slightly higher than the  $T_g$  of the API. This increase in  $T_g$  could be due to the presence of amorphous form of VAL bonded to water, existing outside the CAS, due to the NIC crystallising out, as evidenced by the melting peak endotherm of NIC and NIC peaks in the PXRD pattern in **Figure 6.9 (c)**. Just like the BM CAS stored at 85% RH, it could also be the presence of an amorphous hydrate, whose possibility of occurrence can be supported by the adsorption of moisture as detected in the TGA as shown in **Table 6.5** in section 6.4.2.2. The system aged at 85% RH demonstrates the

presence of three amorphous phases and one crystalline phase of NIC supporting the PXRD findings that the system has undergone phase separation and is inhomogeneous.

The tmDSC data for the SL CAS system stored at 85% RH for 32 weeks shows three glass transition events have been observed in the reversing heatflow thermogram at 49.7 °C, 76.6 °C and 86.7 °C. The total heatflow thermogram shows an endothermic event, with a peak at 92.4 °C, which reflects the evaporation of water from the system, clearly indicating the presence of an amorphous hydrate. The lack of any melting endotherms in the total heatflow suggests that the no crystallinity has been detected. The first  $T_g$  is close to the value of the freshly prepared sample (35.1 °C), indicating that the system is partially stable. However, the occurrence of two additional glass transition events indicates that the system is not entirely homogeneous anymore. The second  $T_g$  at 76.6 °C indicates the presence of an amorphous VAL that has undergone phase separation due to the VAL-NIC bond disruption. The third  $T_g$  at 86.7 °C indicates the potential presence of a VAL hydrate. This clear distinction in  $T_g$ s indicates the presence of both forms of VAL as well as the CAS present in the sample. Furthermore, the presence of moisture in the total heat flow as well as the moisture adsorption TGA thermograms suggests the formation of an amorphous hydrate. The system aged at 85% RH demonstrates the presence of three amorphous phases and an amorphous hydrate and no crystallinity, consistent with the pXRD patterns in [Figure 6.9 \(d\)](#), suggests that the system has undergone AAPS and is inhomogeneous.

The moisture in the surroundings disrupt the bonding between VAL and NIC in a manner where water molecules bind to the two components and forms a VAL-NIC-water system, i.e. a hydrated amorphous system as shown in [Figure 6.14](#), which could potentially alter the  $T_g$  value of the CAS. Hydration will affect NIC, which is smaller than VAL, differently. Due to the hygroscopicity of amorphous NIC as well as its highly polar nature, the amide group of NIC bonds easily with water molecules, which can then insert themselves between the molecules by disrupting the bonding interaction between the components of the CAS, forming a hydrated matrix and enhance the molecular mobility of the excipient and can shift the  $T_g$  of the system to lower value, leading to the onset of phase separation, which ultimately leads to the recrystallisation of NIC.

All the systems were stored at around 20 °C, which is lower than the glass transition value of the drug in both its amorphous and semi-crystalline form as well as the CASs. This would ensure the amorphous stability of the systems as nucleation is expected

to occur at an exceedingly slow rate due to the lower molecular mobility. For the recrystallisation of the CASs, these systems were subjected to an accelerated ageing conditions by storing them under high relative humidity of 85% RH. It was observed that recrystallisation by pXRD was only detected by the excipient NIC, whereas VAL remained amorphous, which leads to the phenomenon of AAPS, as demonstrated by Figure 6.13, where all of the freshly prepared CASs (except MA CAS) have transitioned from having a single  $T_g$  to having multiple  $T_g$ s, indicative of the presence of multiple amorphous phases. Furthermore, based on the tmDSC data where  $T_g$  values are shifted towards both NIC-rich CASs and VAL-rich CASs, the presence of moisture further facilitates the occurrence of AAPS. The recrystallisation of NIC could be attributed to homogeneous nucleation, as it occurs in the absence of any external interference such as impurity or on the walls of the glass, i.e. not surface driven; all of the recrystallisation occurs within the powdered sample itself.

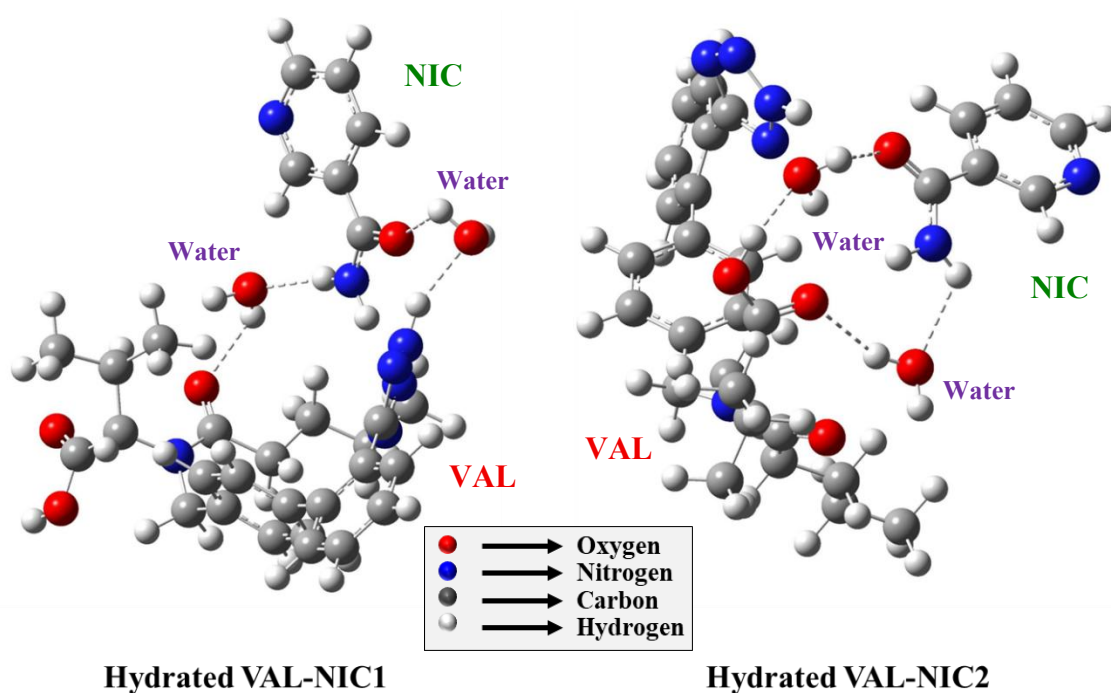


**Figure 6.14:** Simulated structural models of hydrated heterosynthons VAL-NIC 1 and VAL-NIC2, where two molecules of water disrupt the hydrogen bonding interaction within the CAS. The hydrogen bonds are marked with grey dotted lines. Modelled and optimised in GaussView 6.1.1 and Gaussian 09 (235).

From the tmDSC analysis, it is clear that storing CASs stored in a moist environment leads to amorphous-amorphous phase separation in all systems, even in systems without detectable recrystallisation, i.e., AAPS precedes recrystallisation. None of the preparation techniques produce a stable single-phase homogeneous system when

tested against time. Irrespective of humidity, the system undergoes phase separation forming multiple amorphous systems within itself as well as demonstrating the occurrence of both VAL and NIC recrystallisation.

#### 6.4.2.4. Polarised Light Microscopy (PLM)

PLM has been crucial in detecting low levels of crystallinity for all systems stored at both 85% RH and 25% RH. At the lowest humidity of 25% RH, the systems show a small increase of crystallinity after 32 weeks, as shown in [Figure 6.15](#). PLM images of CASs stored at 25% RH for 32 weeks are presented in [Appendix C](#).

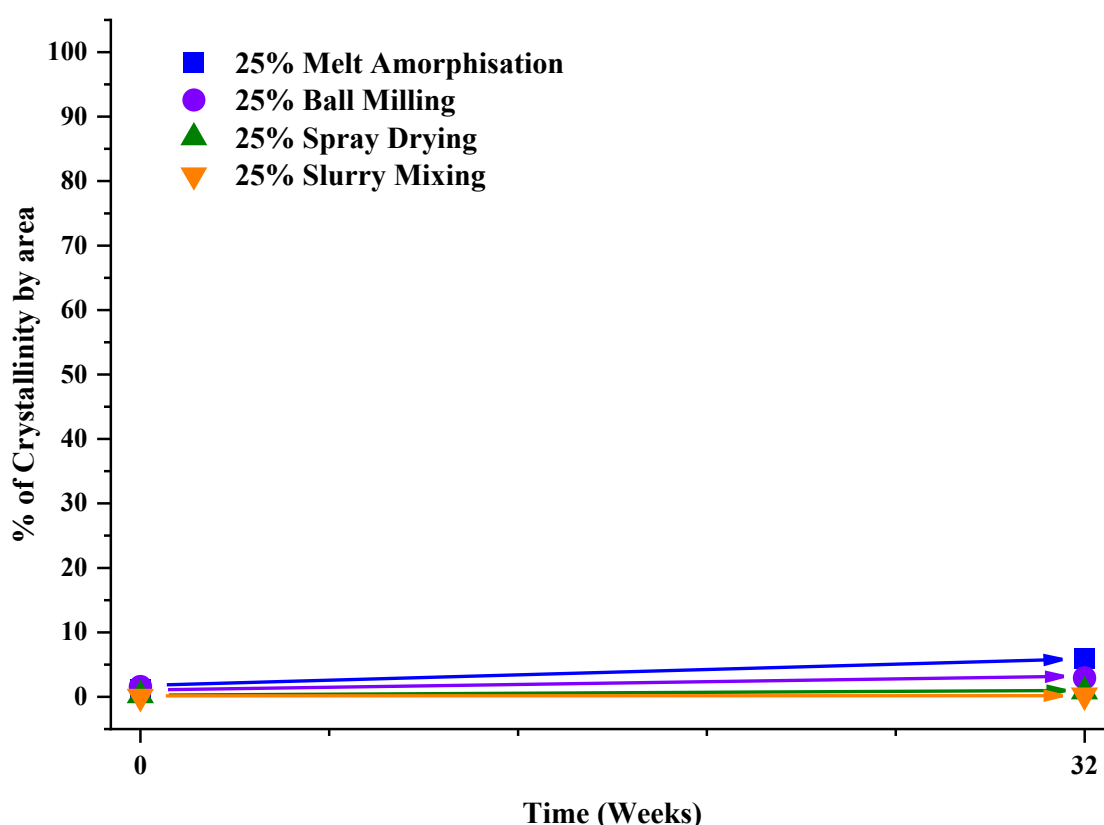


**Figure 6.15:** Increase in crystallinity in MA, BM, SD, and SL CAS at 25% RH over a period of 32 weeks, from PLM images. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size.

The crystallinity rose from 1.1%, 1.6%, 0.2% and 0.0% to 5.9%, 2.9%, 0.8% and 0.2% for MA, BM, SD, and SL CASs, respectively. Although recrystallisation rates are significantly lower when stored at 25% RH than that of storage in 85% RH, phase separation still takes place. This time, it is more prevalent in the systems prepared without solvents. This higher stability in CASs prepared by solvent-based techniques is likely due to the fact that the solution of VAL and NIC is more homogeneous compared to just

milling and melting techniques, with the former systems having better intermolecular bonding between the two components whilst the latter have free VAL or free NIC in the system, unbonded to each other, which has already been showcased by the BM CAS in [Chapter 5](#).

However, it is quite clear that the system does recrystallise and the recrystallisation is performed only by NIC. [Figure 6.16](#) shows the particles of MA, BM, SD, and SL CASs at weeks 0 and 32 of storage at 85% RH and are bordered in blue, purple, green and orange, respectively. When stored at 85% RH, the frequency of crystalline particles in the CASs rapidly increases over time. [Figure 6.17](#) demonstrates the increasing crystallinity in the CASs over a period of 32 weeks when stored at 85% RH, by obtaining a polynomial fit of order 2 by analysing the crystallinity (by area) of the particles in 10 images at every two weeks for all four systems. These crystallites are reflective of NIC and not VAL, as it is the excipient that recrystallises in the form of needles. From week 0 to week 32, the percentage of crystallinity increases from 1.1 % to 50.4%, 0.2% % to 60.2% and 0.0% to 23.1% for the MA, SD, and SL CASs respectively, with no crystallinity of VAL detected. This significant increase in crystallinity suggests that moisture preferentially facilitates in the nucleation of NIC molecules. At 85% RH, the BM CAS, it started with around 1.5% crystallinity, which gradually rises to 25% after 26 weeks in the ageing chamber and then appears to have plummeted to 6.6% at 32 weeks. Although the overall percentage of crystallinity has increased, but the fluctuations in the trend could be a result of the lack of uniformity in the BM CAS. This lack of homogeneity in the ball milled CAS could lead to sporadic nucleation in the system where the presence of NIC crystallites is aggregated more in one part of the system than the other. The second reason could be issue of sampling, where the system under the microscope just happened to have less amount of crystallinity due to the lack of NIC crystallites in that area.

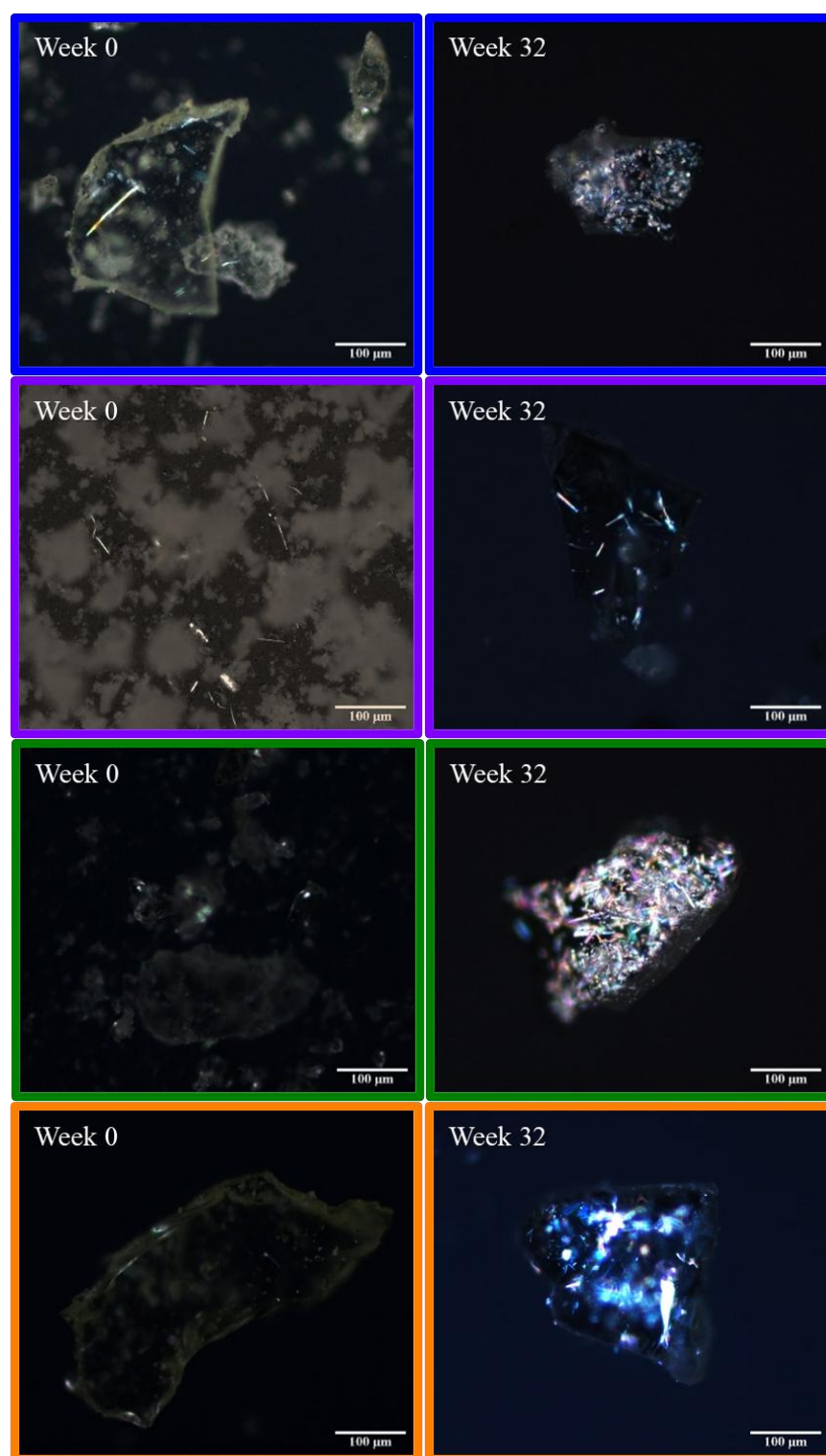


**Figure 6.16:** PLM images of MA, BM, SD, and SL CASs (bordered in blue, purple, green and orange respectively) weeks 0 and 32 at 85% RH. are Crystallinity was evaluated by analysing 10 images per sample, at a given time.

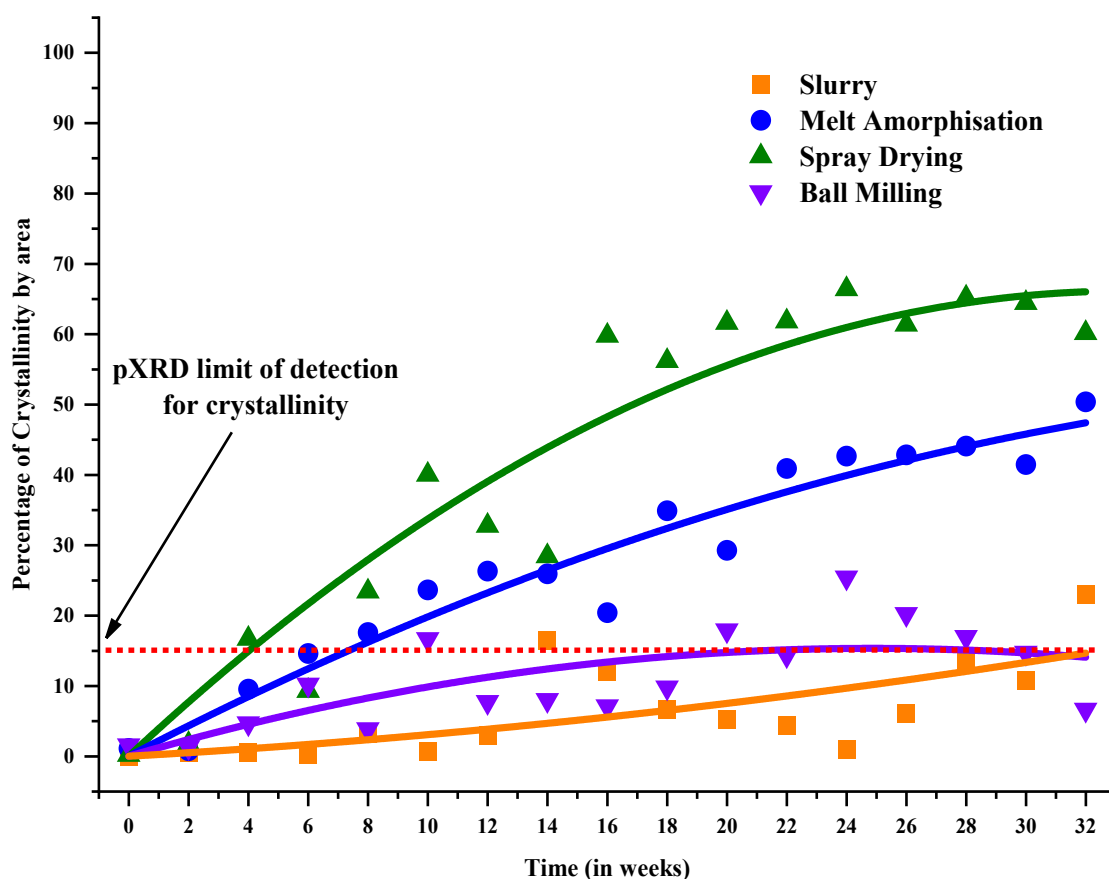


**Figure 6.17:** Trend in crystallinity in the CASs at 85% RH over a period of 32 weeks, from PLM images. Polynomial fit (order 2) was taken by estimating the crystallinity by area of the particles of 10 images at every fortnight for all four systems. The limit of detection of crystallinity by pXRD is approximately 15% of the area imaged by PLM. It is important to note that these values correspond to the size of the sample size provided (which in this case was 10), and it does not reflect true crystallinity of the systems; these values are likely to increase or decrease with the increase in sample size.

The amorphous powder has adsorbed moisture from the surroundings, especially at the high humidity of 85% RH, where VAL and NIC bonds are disrupted and causing phase separation. This migration of NIC molecules appears to occur within the amorphous particles, causing NIC crystallites to nucleate within particles. This formation of a stable nucleus cluster starts to occur within 4 weeks of ageing for the MA and SL CASs, and by week 8, the percentage of crystallinity are 17.6% and 23.4% respectively, whilst the percentage of crystallinity for the BM and SL CASs are 3.8% and 3.2% respectively at the same time. This growing trend of NIC crystallinity does not affect VAL, which is expected as the crystalline nucleation of VAL growth does not occur rapidly in both its semi-crystalline and amorphous form.

Figure 6.17 shows that the SD CAS has the highest crystallinity of 60% followed by the MA, BM, and SL CASs, where these three systems possessed crystallinity below

60%, despite all four systems were stored at 85% RH. The high crystallinity in the SD CAS can be assigned to the water molecules present in the binary system, as it was prepared from a ethanol-water solvent mixture, which enhances the recrystallisation tendency of the NIC present in the amorphous particles, letting it achieve around 60% crystallinity after the end of the ageing study in at 85% RH. The MA CAS is next in terms of recrystallisation rate, it did not have trapped water molecules present in the system which could have facilitated the recrystallisation but the clustering of unbonded NIC throughout the system could be a reason for the high levels of crystallinity. When exposed to moisture, these NIC molecules have undergone nucleation to form crystallites in the system, accounting for approximately 50% crystallinity at the end of the ageing study. BM CAS was the next system that comes after MA CAS in terms of recrystallisation after 32 weeks of ageing. It has considerably low levels of crystallinity, at around 16%. This is interesting as the BM CAS was phase separated prior to the ageing study, and it would have been expected to have higher crystalline content, but PLM data suggested otherwise. The last system to show elevated recrystallisation is SL CAS. It is has demonstrated highest stability against recrystallisation for the longest time. Crystallinity is just around 15% after the 32 weeks of storage, which is considerably lower than all the other CASs.

The limit of detection for a typical laboratory X-ray diffractometers for pXRD is approximately 5 vol%, which translates to 1 to 5 wt% under preferred crystal orientation and density differences. From [Figure 6.17](#), an inferred detection limit for pXRD has been obtained from crystalline area measurements in PLM. It is clear that PLM is more sensitive than pXRD for detecting crystallinity. pXRD was able to detect crystalline NIC needles when they occupy around 15% of the area of powder when imaged by PLM. In contrast, PLM can detect crystal needles to 0.2% of the area of the powdered samples during imaging.

Despite the differences in moisture adsorption, bulk crystallinity, and multiple amorphous phases, all of the systems have demonstrated a gradual increase in crystallinity when stored at 85% RH for a period of 32 weeks. It is important to note that rate of increase in crystallinity varies significantly for MA and SD CASs when compared to the SL and BM CASs. This variability can be attributed to the different modes of preparation as well as the inhomogeneity within the CASs. When compared together, it does appear that the SL CAS is the most ideal system in terms of overall stability against recrystallisation under high humidity.

It can be concluded that the CASs tend to undergo phase separation and recrystallisation more readily at 85% RH than 25% RH for the same period, as the excess of moisture in the surroundings disrupt the hydrogen bonding interaction between VAL and NIC.

#### 6.4.2.5. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy has been employed on the CAS to study the influence of moisture on the drug when stored at different levels of humidity for a period of 32 weeks. [Figure 6.18](#) shows the FT-IR spectra of CASs stored in 85% RH after 32 weeks. The shifts in the vibrational peaks of the functional groups involved in hydrogen bonding for each of the systems are listed in [Table 6.8](#). It is clear from the spectra that systems stored at 85% RH have absorbed significantly more water after 32 weeks, as is expected. The systems stored at 25% RH has not undergone any significant changes nor have they absorbed any significant moisture that affects their peak intensities. FT-IR spectra for CASs stored at 25% RH after 32 weeks are presented and tabulated in [Appendix C](#).

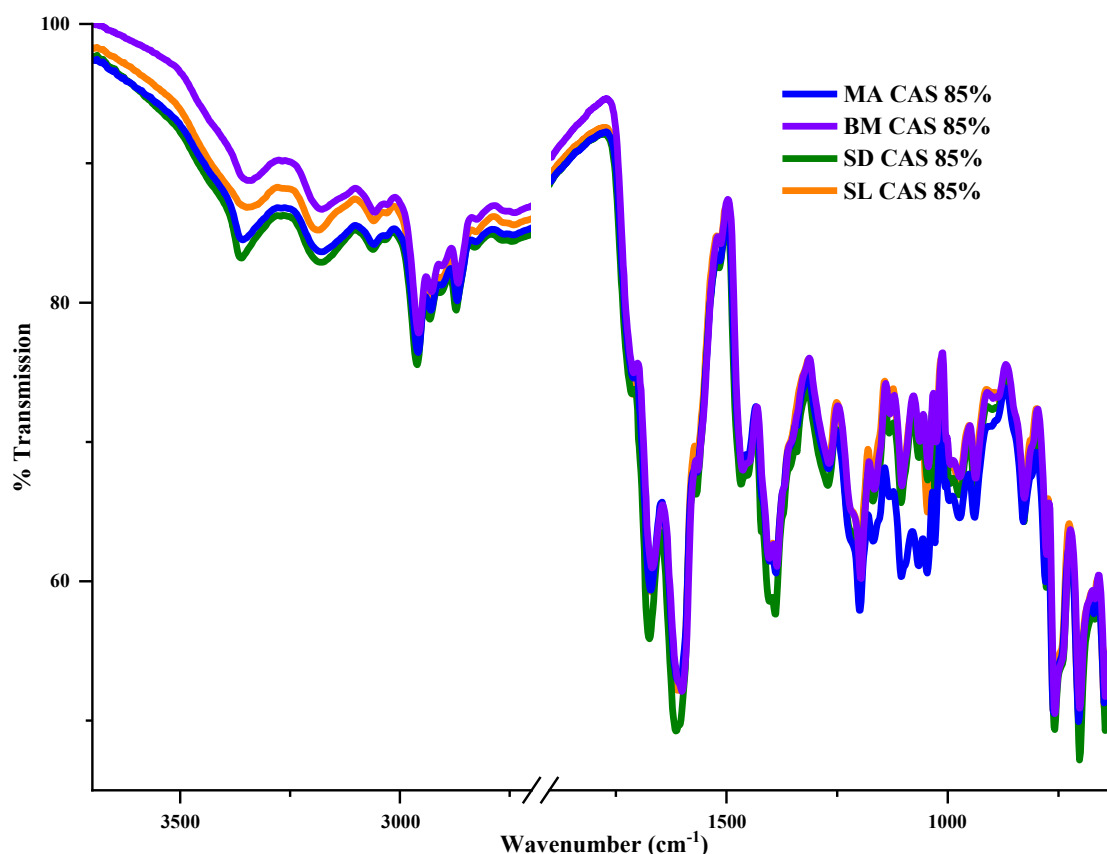


**Figure 6.18:** FT-IR spectra of the MA, BM, SD, and SL CASs at 85% RH at week 32.

Phase separation has occurred across all the CASs at stored low relative humidity as evidenced by the shift in peak positions, similar to the shifts in CASs when stored at higher humidity. At 25% RH, major changes were observed in the MA CAS. The OH bond of VAL for the BM, SD and SL CASs were around  $3185\text{ cm}^{-1}$ , similar to that of the systems stored at 85% RH but for the MA CAS, the peak was at  $3205\text{ cm}^{-1}$ . This suggests that the phase separation does not occur uniformly through the system, and bonding interactions are still maintained. The unreacted and reacted asymmetric NH and symmetric peaks for the BM, SD and SL CASs are present at around  $3336\text{ cm}^{-1}$ ,  $3185\text{ cm}^{-1}$ ,  $3061\text{ cm}^{-1}$  respectively, but are at  $3356\text{ cm}^{-1}$ ,  $3205\text{ cm}^{-1}$ , and  $3078\text{ cm}^{-1}$  respectively for the MA CAS. The NH bending vibrations for the BM, SD and SL CASs have remained unchanged but has moved to  $1620\text{ cm}^{-1}$  and  $1583\text{ cm}^{-1}$  for the MA CAS, a value closer to the original NH bending values. Similarly, the C=O peaks of acid and amide of VAL and amide of NIC are present at  $1714\text{ cm}^{-1}$ ,  $1603\text{ cm}^{-1}$  and  $1670\text{ cm}^{-1}$  for the BM, SD and SL CASs but are present at higher values of  $1730\text{ cm}^{-1}$ ,  $1620\text{ cm}^{-1}$  and  $1686\text{ cm}^{-1}$  for the MA CAS. These higher shifts in the MA CAS are most likely due to a higher extent of phase separation in the system compared to rest of the CASs. The lack of tetrazolyl peak across all four CASs indicates that the tetrazolyl H is still involved in hydrogen bonding interaction in the system. The MA CAS has exhibited higher NIC crystallinity compared to the other CASs when stored at 25% RH, as evidenced by the PLM data presented in [Figure 6.17](#) and presence of the VAL crystallinity due to the presence of a small melting endotherm of VAL presented in [Figure 6.11](#). This would suggest that phase separation is more commonplace for the MA CAS than any other CAS and the higher shifts in the peaks compared to other CASs supports the idea even further.

**Table 6.8:** Experimental Values of FT-IR spectra for MA, BM, SD, and SL CASs at 85% RH at week 32 along with the shifts ( $\Delta$ ) in values of CASs prior to ageing (fresh).

| <b><u>Functional Groups</u></b>     | <b><u>Frequency (cm<sup>-1</sup>)</u></b>  |                                  |                                            |                                            |
|-------------------------------------|--------------------------------------------|----------------------------------|--------------------------------------------|--------------------------------------------|
|                                     | <b><u>MA CAS</u></b>                       | <b><u>BM CAS</u></b>             | <b><u>SD CAS</u></b>                       | <b><u>SL CAS</u></b>                       |
| (O-H) stretching                    | 3182 ( $\Delta=+8$ )                       | 3192 ( $\Delta=+9$ )             | 3180 ( $\Delta=-2$ )                       | 3192 ( $\Delta=+11$ )                      |
| $\nu(\text{NH})_{\text{tetrazole}}$ | -                                          | -                                | -                                          | -                                          |
| Acid (C=O) stretching               | 1714 ( $\Delta=-3$ )                       | 1715 ( $\Delta=+2$ )             | 1715 ( $\Delta=+2$ )                       | 1715 ( $\Delta=+1$ )                       |
| Amide (C=O) stretching              | 1605<br>(Shoulder at 1614) ( $\Delta=+2$ ) | 1603<br>(Shoulder at 1616)       | 1605 ( $\Delta=+2$ )<br>(Shoulder at 1614) | 1606<br>(Shoulder at 1614) ( $\Delta=+3$ ) |
| Unreacted (N-H) as. stretching      | 3360 ( $\Delta=+2$ )                       | 3356 ( $\Delta=-20$ )            | 3361 ( $\Delta=+24$ )                      | 3348 ( $\Delta=-8$ )                       |
| (N-H) as. stretching                | 3182 ( $\Delta=+8$ )                       | 3192 ( $\Delta=+9$ )             | 3180 ( $\Delta=-2$ )                       | 3192 ( $\Delta=+11$ )                      |
| (N-H) stretching                    | 3061 ( $\Delta=+1$ )                       | 3065 ( $\Delta=+4$ )             | 3061 ( $\Delta=-1$ )                       | 3063 ( $\Delta=+1$ )                       |
| (C=O) stretching                    | 1674 ( $\Delta=+21$ )                      | 1672 ( $\Delta=+4$ )             | 1674 ( $\Delta=+6$ )                       | 1672 ( $\Delta=+7$ )                       |
| (N-H) bending                       | 1605, 1568<br>( $\Delta=+2, +1$ )          | 1603, 1566<br>( $\Delta=0, -1$ ) | 1605, 1566<br>( $\Delta=+2, -1$ )          | 1606, 1568<br>( $\Delta=+3, +1$ )          |

In the CASs stored at 85% RH, the peak intensities of both asymmetric and symmetric stretching vibrations of NH peaks, have shifted slightly from the freshly prepared CASs on ageing. At 85% RH, the unreacted asymmetric NH stretching vibrations have remained unshifted at 3358 cm<sup>-1</sup> for the MA CAS but has increased to 3361 cm<sup>-1</sup> for the SD CAS but decreased to 3356 cm<sup>-1</sup> and 3348 cm<sup>-1</sup> for the BM and SL CASs. The asymmetric stretching of NH peaks involved in hydrogen bonding is present within 3180 cm<sup>-1</sup> to 3192 cm<sup>-1</sup> for all four CASs. The NH vibrations for all four systems are present around the 3062 cm<sup>-1</sup> with small shifts from their original spectra (prior to ageing). This shift in the peak positions suggests possible phase separation where some NIC molecules are overcoming their hydrogen bonding interactions with VAL and

moving towards to the original peak positions of free NIC. Additionally the NH bending vibrations for all the CASs have undergone a very small increase, which further supports that the NIC molecules are overcoming their hydrogen bonding interactions. Furthermore, the peak intensities have shown a significant increase for the systems when stored at 85%. This increase in peak intensity can be attributed to moisture as H<sub>2</sub>O demonstrates a broad absorbance peak within the range of 2700 to 3700 cm<sup>-1</sup>, caused by the symmetric and asymmetric stretching of the OH bonds. The peak position for the OH bond is now present between 3180 cm<sup>-1</sup> to 3192 cm<sup>-1</sup> for the CASs. This shift in the peak maybe influenced by the moisture adsorbed by the binary system, which is supported by the increase in peak intensities for the OH stretching vibration, suggesting that this minor change is caused by moisture adsorption.

For the C=O groups, the peak intensities have increased, same as the NH peaks. This increase also could be due to the adsorption of moisture. The C=O<sub>acid</sub> of VAL peaks have shifted to around 1715 cm<sup>-1</sup> for the BM, SD, and SL CASs and to 1713 cm<sup>-1</sup> for the MA CAS. Such small changes in the opposite direction may not suggest phase separation but could be indicative of hydrogen bonding interactions with either NIC or adsorbed water. The C=O<sub>amide</sub> group for VAL in the CAS has shifted from 1603 cm<sup>-1</sup> to 1605 cm<sup>-1</sup> for the MA, SD, and SL CASs, and for the BM CAS, it stayed at 1603 cm<sup>-1</sup>. This C=O<sub>amide</sub> group has not shown any shift towards the original value of VAL at 85% RH, but the shift to a higher value for the lower humidities further support the concept of phase separated VAL across all the CASs. The C=O<sub>amide</sub> group for NIC in the CASs have increased from to around 1673 cm<sup>-1</sup>. The increasing shift in the C=O<sub>amide</sub> for NIC in CASs is indicative of phase separation as these values are moving closer to the original value of amide group of NIC.

The increase in the intensity and shifts in the NH and C=O stretching peaks is indicative of the presence of adsorbed moisture in the CASs, as also evidenced by TGA in section 6.4.2.2. This potentially leads to moisture-aided phase separation in the CAS stored at 85%, supported by the findings of tmDSC as seen in section 6.4.2.3. However, only under high humidity was NIC crystallinity detected, suggesting that high moisture content is fundamental in the recrystallisation of the excipient. A lower moisture content would only initiate phase separation. This is proven when the CASs are stored at 25% RH, temporal phase separation takes place, suggesting that phase separation occurs even in the absence of water in the co-amorphous systems.

#### 6.4.2.6. Raman Spectroscopy

Raman spectroscopy has been employed on the CASs to study non-polar interactions within the co-amorphous systems, something that the FT-IR does not readily detect. Raman spectroscopy is sensitive to the framework of moisture induced hydrogen bonding networks and any of the minor changes in the peak positions may be attributed to the rearrangement in the binding interaction between the two components due to the intrusion of water molecules, which could lead to the formation of amorphous hydrates, leading to phase separation (279). [Figure 6.19](#) and

demonstrate the changes in the Raman spectra of the CASs to study the influence of moisture on the CAS when stored at 85% RH for a period of 32 weeks. Raman spectra for CASs stored at 25% RH after 32 weeks are presented and tabulated in [Appendix C](#).

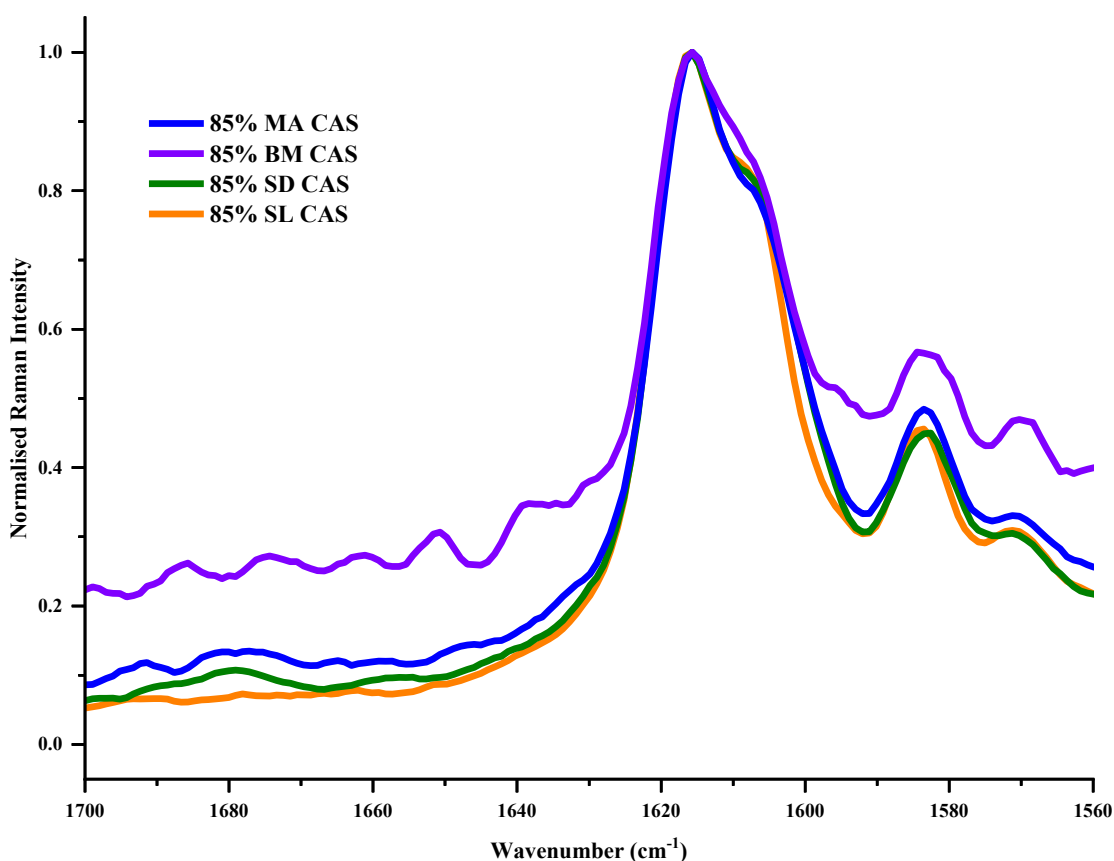


**Figure 6.19:** Raman spectra of MA, BM, SD, and SL CASs at 85% RH at week 32.

At 25% RH, the peak of C=O<sub>amide</sub> of VAL were at 1613 cm<sup>-1</sup>, 1610 cm<sup>-1</sup> for the MA and BM CASs respectively and 1615 cm<sup>-1</sup> for the SD and SL CASs. The shift to the same value in SD and SL CASs could be due to the fact that these two systems have not undergone phase separation as much as the other two CASs, which has been evidenced by PLM analysis. The peak at 1607 cm<sup>-1</sup> of crystalline NIC, assigned to the vibration of

the N-H bonds remained unchanged for the MA, SD, and SL CASs but the BM CAS shows a decrease of  $10\text{ cm}^{-1}$ . The decrease in the peak for the BM CAS stored at 25% RH may suggest that the shift was due to the disruption of hydrogen bonds between VAL and NIC, possibly forming an intermolecular hydrogen bond between two NIC molecules (N-H---H-N). This would suggest the occurrence of phase separation with the NIC existing by itself in the binary mixture, as evidenced by tmDSC data in [Figure 6.11](#). However, the N-H bending vibration of VAL showed a slight decrease to  $1568\text{ cm}^{-1}$  for the MA CAS (moving towards the original peak position at  $1564\text{ cm}^{-1}$ ) but unchanged for the other CAS which could be indicative of the disruption of hydrogen bonding between the tetrazolyl H of VAL and carbonyl C=O group of NIC leading to phase separation, with the VAL existing by itself in the binary mixture, as evidenced by the appearance of VAL's melting point at  $108.93\text{ }^{\circ}\text{C}$  in the tmDSC data depicted in [Figure 6.11](#). Furthermore, the C=C stretching vibration only decreases for MA CAS, from  $1570\text{ cm}^{-1}$  to  $1568\text{ cm}^{-1}$  and remains unshifted for other CASs. This red shift could be due to the self-aggregation of NIC due to the stacking of pyridine rings of NIC by  $\pi$ - $\pi$  interactions (31, 135).

All four CASs show a characteristic peak at  $1616\text{ cm}^{-1}$  corresponding to  $\text{C}=\text{O}_{\text{amide}}$  stretching vibrations of VAL in the systems stored at 85% RH. The shift in the wavenumber from  $1615\text{ cm}^{-1}$  in the MA, SD and SL CASs and from  $1611\text{ cm}^{-1}$  in the BM CAS to  $1616\text{ cm}^{-1}$  at 85% RH, suggests the formation of additional hydrogen bonding interaction due to moisture. The N-H bending vibration of VAL has shown an increase of  $1\text{ cm}^{-1}$  for all the systems stored at 85% RH. This increase would indicate the formation of a hydrogen bond, as observed in [Chapter 5](#). As moisture disrupts the hydrogen bonding interaction between VAL and NIC, it can be said that this new bond of VAL is possibly with water, potentially forming a hydrate. During the formation of the CASs except the SD CAS, the  $\text{C}=\text{O}_{\text{amide}}$  of NIC goes undetected. However, as the moisture content surrounding it increases and a small broad peak appears at  $1679\text{ cm}^{-1}$  for the SD CAS when stored at 85% RH. This peak is the stretching frequency of  $\text{C}=\text{O}_{\text{amide}}$  of NIC and occurs in pure NIC. This resurgence of the  $\text{C}=\text{O}_{\text{amide}}$  peak in SD CAS suggests the presence of unbonded NIC existing in the SD CAS at high humidity, suggesting phase separation, which further supports the findings of pXRD and PLM in [Figure 6.9 \(c\)](#) and [Figure 6.17](#). The N-H bending vibration of crystalline NIC at  $1606\text{ cm}^{-1}$ ,  $1611\text{ cm}^{-1}$ ,  $1608\text{ cm}^{-1}$  and  $1606\text{ cm}^{-1}$  in the MA, BM, SD, and SL CASs has shifts to  $1607\text{ cm}^{-1}$  when stored at 85% RH and the other NH bending vibration remains undetected. The shift in

the N-H bending vibration for 85% RH may suggest that the shift was due to the formation of hydrogen bonds, likely as a hydrate.

**Table 6.9:** Experimental Values of Raman spectra for MA, BM, SD, and SL CASs at 85% RH at week 32 along with the shifts ( $\Delta$ ) in values of CASs prior to ageing (fresh). Stretching and bending interactions are demoted by the suffices s and b respectively and UD indicates that the peaks go undetected.

|                   |                             | <u>Wavenumber (cm<sup>-1</sup>)</u>                 |                                                  |                                                |                                                 |
|-------------------|-----------------------------|-----------------------------------------------------|--------------------------------------------------|------------------------------------------------|-------------------------------------------------|
| <u>Components</u> | <u>Functional Groups</u>    | <u>MA CAS</u>                                       | <u>BM CAS</u>                                    | <u>SD CAS</u>                                  | <u>SL CAS</u>                                   |
| VAL               | C=O<br>(amide) <sub>s</sub> | 1616 (+1)<br>shoulder at<br>1607                    | 1616 ( $\Delta=+5$ ),<br>shoulder at<br>1607     | 1616<br>( $\Delta=+1$ )<br>shoulder at<br>1608 | 1616 ( $\Delta=+2$ )<br>shoulder at<br>1609     |
|                   | N-H <sub>b</sub>            | 1571<br>( $\Delta=+1$ )                             | 1570 ( $\Delta=+1$ )                             | 1571<br>( $\Delta=+1$ )                        | 1570 ( $\Delta=+1$ )                            |
|                   | C=C <sub>s</sub>            | 1571<br>( $\Delta=+1$ )                             | 1570 ( $\Delta=+1$ )                             | 1571<br>( $\Delta=+1$ )                        | 1570 ( $\Delta=+1$ )                            |
| NIC               | C=O<br>(amide) <sub>s</sub> | UD                                                  | UD                                               | 1679<br>( $\Delta=+2$ )                        | UD                                              |
|                   | N-H <sub>b</sub>            | UD, 1607<br>(merged<br>with VAL)<br>( $\Delta=+1$ ) | UD, 1607<br>(merged with<br>VAL) ( $\Delta=-4$ ) | UD, 1608<br>(merged<br>with VAL)               | UD, 1609<br>(merged with<br>VAL) ( $\Delta=3$ ) |
|                   | C=C <sub>s</sub>            | 1571<br>( $\Delta=+1$ )                             | 1570 ( $\Delta=+1$ )                             | 1571<br>( $\Delta=+1$ )                        | 1570 ( $\Delta=+1$ )                            |

C=C stretching vibrations have increased by 1 cm<sup>-1</sup> for all CASs to 1570 cm<sup>-1</sup> for the BM and SL CASs and 1571 cm<sup>-1</sup> for the MA and SD CASs when stored at 85% RH. This red shift can possibly indicate a disruption in the  $\pi$ - $\pi$  stacking interaction between

the VAL and NIC molecules, as water molecules can get between the aromatic rings, and thus disforming the stacking (135). With the increase in the frequency of the C=O<sub>amide</sub> group of VAL along with the disruption of the  $\pi$ - $\pi$  stacking in the CASs stored at 85% RH, it can be said that moisture present in the system, as evidenced by TGA, is causing a phase separation in the CASs. Furthermore, the reappearance of C=O<sub>amide</sub> group of NIC at 85% RH for the SD CAS indicated that this two CAS is more phase separated with the recrystallisation of NIC being more prominent, thus supporting the findings of pXRD, tmDSC, PLM and FT-IR. However, it can also be suggested that an amorphous hydrate is present, as evidenced by the increase in the bending vibration in the tetrazolyl H of VAL. The amorphous hydrate can exist sporadically throughout the system as the Raman spectra does not demonstrate any massive shifts in the system other than demonstrating the potential presence of bonded water in the system.

CASs stored at 25% RH do not demonstrate any evidence of hydrogen bond formation but do have signs of hydrogen bond disruption between VAL and NIC. However, this disruption can only be observed in the MA and BM CASs indicating the occurrence of phase separation in the absence of moisture, with the SL and SD CASs remaining unchanged during the 32 week of storage, further supporting the findings of the PLM, where the CAS prepared by solvent-based techniques have higher stability against kinetic phase separation.

#### 6.4.2.7. Summary of Case Study II

Temporal phase separation occurs in the systems stored at 25% RH which later leads to recrystallisation. Although the systems were X-ray amorphous for 32 weeks, amorphous-amorphous phase separation was observed due to multiple glass transition events and appearance of melting endotherms. Small levels of recrystallisation were detected by PLM, but it was mostly the MA CAS. The SD and SL CASs did not show any significant rise in crystallinity. Spectroscopic data observed the bond disruption between the components, especially in the MA CAS, thus supporting the findings of PLM. Phase separation and low levels of recrystallisation was detected by tmDSC in the CASs indicate that the VAL-NIC system would undergo phase separation even under dry conditions if enough time passes, i.e., temporal phase separation

Out of the four binary formulations, the slurry mixed CAS is the most stable against recrystallisation as it maintained its X-ray amorphous state for 32 weeks when stored at 85% RH. The melt amorphised CAS and the spray dried CAS recrystallised after

4 weeks in the humidity chamber of 85% RH. However, phase separation was detected in all four CASs, as evidenced by the presence of multiple glass transition temperatures and the appearance of melting endotherms. This suggests that amorphous-amorphous phase separation takes place along with small scale recrystallisation occurring within the system when stored in high humid environment for 32 weeks. PLM observed recrystallisation across all four systems, with the highest crystallinity (by area) was for the SD CAS, going up to 66% and the lowest for the SL CAS, with a small rise to 23%, indicating that the SL CAS is most resilient against recrystallisation under high humidity. This phase separation has been observed by the changes in peak positions in FT-IR and Raman spectroscopy, which demonstrates the bond disruption between VAL and NIC in the CASs along with the possible formation of amorphous hydrates within the system. This occurrence of amorphous hydrates is a possibility when stored at 85% RH, when the systems are subjected to a moisture-laden environment.

### 6.4.3. Equilibrium Solubility

The main goal of formulating co-amorphous solid drug delivery systems is improving solubility of poorly soluble APIs. In the previous chapter, we had observed the significant increase in the solubility of VAL in the CASs with NIC, for all four systems. [Figure 6.20](#) and [Table 6.10](#) demonstrate the changes equilibrium solubility of the systems stored at 85% RH and 25% RH (only shown in [Table 6.10](#)) after 4 weeks of ageing.

The systems stored at 25% RH showed a very small decrease in equilibrium solubility with respect to the freshly prepared systems. The equilibrium solubility for each of these systems can be averaged to  $5.79 \text{ mg mL}^{-1}$ , which is a 3.3% decrease from when they were first prepared. This suggests that after 4 weeks of storage in low humidity, the systems have not undergone any significant changes that can impact its performance, as evidenced by the lack of moisture adsorption and little to no recrystallisation during that duration. The solubility of VAL in both its forms is still the same during this time period, at this humidity level. This shows that co-amorphous formulations retain their high performance, compared to the API at low humidity levels.

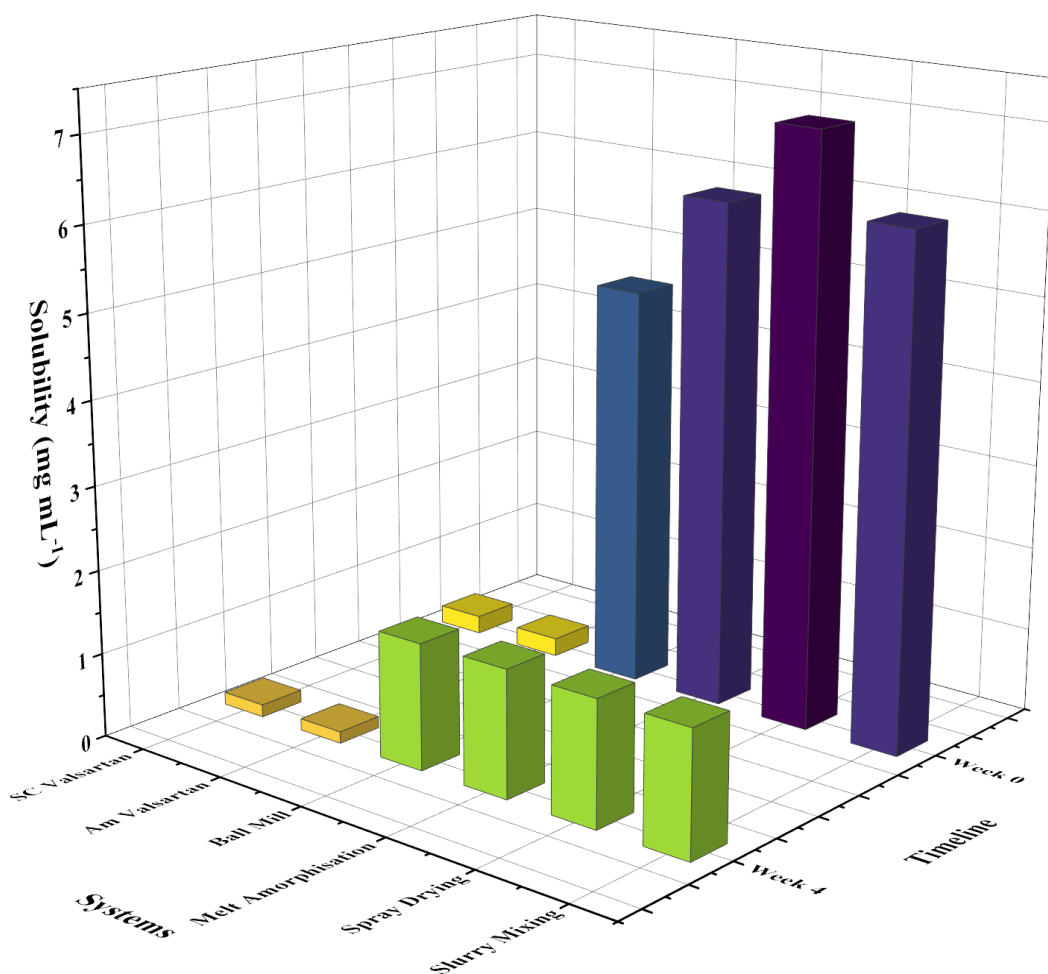


**Figure 6.20:** Changes in equilibrium solubility after 4 weeks of ageing at 85% RH. Each experimental data is an average of three repeats.

The equilibrium solubility for each of the freshly prepared sample can be averaged to  $5.99 \text{ mg mL}^{-1}$  with a standard deviation of  $0.92 \text{ mg mL}^{-1}$ , with the CAS having the highest equilibrium solubility being the SD CAS at  $7.04 \pm 1.97 \text{ mg mL}^{-1}$  and the one with the lowest equilibrium solubility being the BM CAS at  $4.80 \pm 2.14 \text{ mg mL}^{-1}$ . However, after 4 weeks in the humidity chamber (85% RH), the equilibrium solubility has dropped to an absolute value of  $1.51 \pm 0.01 \text{ mg mL}^{-1}$  for all four CASs, despite their different modes of preparation and their different stability against recrystallisation under high humidity. Even the solubility of VAL, both amorphous and semi-crystalline has fallen to  $0.15 \pm 0.01 \text{ mg mL}^{-1}$ , suggesting that moisture diminishes solubilising capability. This suggests that the performance of the drug is independent of the stability against recrystallisation of NIC under high humidity. However, the question arises, why do all the CASs perform in the same manner under high humidity despite their different methods of preparation and their different stability against recrystallisation?

**Table 6.10:** Aqueous solubilities of SC VAL, Am VAL and co-amorphous formulations in before and after ageing at 85% and 25% RH. Each value is an average of three repeats along with a standard deviation.

|                    | <u>Solubility (mg mL<sup>-1</sup>)</u> |                               |                               |
|--------------------|----------------------------------------|-------------------------------|-------------------------------|
| <u>Systems</u>     | <u>Freshly prepared systems</u>        | <u>Aged systems at 85% RH</u> | <u>Aged systems at 25% RH</u> |
| Valsartan          | 0.22± 0.22                             | 0.15±0.01                     | 0.21±0.09                     |
| Am Valsartan       | 0.22± 0.05                             | 0.14±0.01                     | 0.22±0.01                     |
| Ball Mill          | 4.80±2.14                              | 1.51±0.02                     | 4.48±1.04                     |
| Melt Amorphisation | 6.05±1.15                              | 1.52±0.00                     | 5.95±0.39                     |
| Spray Drying       | 7.04±1.97                              | 1.50±0.01                     | 6.92±0.29                     |
| Slurry             | 6.10±0.65                              | 1.50±0.01                     | 5.80±0.25                     |

The solubility of the CASs appears to be driven by NIC, as it has a high aqueous solubility than VAL (1 g mL<sup>-1</sup> vs 2.2 x 10<sup>-5</sup> g mL<sup>-1</sup> respectively), due to its hydrotropic nature (14, 31, 108, 243). Upon bonding with the NIC in the freshly prepared CASs, the VAL molecule overcomes its interaction with other VAL molecules; in water, NIC dissolves rapidly in the process, breaks the secondary hydrogen bonds to VAL, to disrupt the tetrahedral network of water molecules thus creating a cavity to enhance the interaction between VAL and water, whilst maintain the  $\pi$ - $\pi$  interaction with VAL, thereby enhancing VAL solubility. However, the dip in solubility during the ageing process is potentially due to the formation of an amorphous hydrate. Physical evidence of an amorphous hydrate is that the systems appear sticky or clumped up together instead of the white powder prior to storage in high humidity, as evidenced by the increase in  $T_g$  values that is potentially due to the API bonding with water as seen in sections 6.4.2.2 and 6.4.2.3. Such an obvious change in physical properties would affect the structural arrangement of the systems. The moisture disrupts the hydrogen bonding interaction and can possibly disrupt the  $\pi$ - $\pi$  interaction between the two components as a water molecule is small enough move between the aromatic rings stacked together and disrupt that additional secondary reaction and potentially forms a VAL-(H<sub>2</sub>O)<sub>2</sub>-NIC network in the

co-amorphous system in both heterodimers, as demonstrated in [Figure 6.14](#) (146, 180, 181).

Disruption of the secondary bonding interactions between the two components, due to the insertion of water molecules, would diminish the solubilising effects of NIC, where NIC would dissolve on its own, leaving the VAL behind, to also dissolve in water or precipitate. In the absence of the water molecule network within the heterosynthons, the performance was enhanced as seen in [Chapter 5](#). Furthermore, the potential formation of an amorphous hydrate adds a new dimension to the CASs, altering the structural disarrangement that is present in the binary amorphous system, as it requires the two components to be arranged in a unique heterosynthons(s) that includes water molecules. This new arrangement might be enhancing the energy of the CAS, which makes it difficult for the drug to disintegrate and dissolve in the water compared to the freshly prepared anhydrous CAS. Now, the solubility of the CASs has not dropped to the absolute value of the drug itself. This might be due to the sporadic nature of the amorphous hydrate, which is not present in the systems uniformly. Some amount of VAL may still be bonded to NIC, as evidenced by the unchanged  $T_g$  amongst other  $T_g$ s in the multi-phased system, and these unbroken VAL-NIC dimers are dissolving in the same manner as the freshly prepared samples. The only difference is that the quantity of such heterosynthons may be low, which is reflected by the absolute solubility of all the CASs.

It is worth noting that the performance of the CASs after ageing is the same irrespective of their modes of preparation and their stability against recrystallisation. This would suggest that recrystallisation is not the driving force for the destabilisation and the downturn in the performance of the drug delivery systems. It is the disruption of the hydrogen bonding interaction along with the disruption of  $\pi$ - $\pi$  interaction due to the formation of amorphous hydrates and the phase separation of the systems that affects the performance of these systems.

#### 6.4.4. Nucleation

Thermal undercooling is the thermodynamic driving force for the generation of a crystal nucleus. It is the phenomenon where a liquid is cooled to a temperature below its melting point without solidifying, thus creating a metastable state. This allows for the increased diffusion which enhances the chances of forming a stable crystal nucleus. All the systems were stored at around 20 °C, which is lower than the glass transition value of the drug in both its amorphous and semi-crystalline form as well as the CASs. This would

ensure the amorphous stability of the systems as nucleation is expected to occur at an exceedingly slow rate due to the lower molecular mobility due to increased viscosity where the system is essentially frozen like a glass. Yet these systems do recrystallise when CASs were subjected to an accelerated ageing by storing them under high relative humidity of 85% RH. It was observed that recrystallisation was only carried out by the excipient NIC, whereas VAL remained amorphous, which leads to the phenomenon of AAPS, as demonstrated by [Figure 6.11](#), where all of the freshly prepared CASs have transitioned from having a single homogeneous phase to having multiple  $T_g$ s, indicative of the presence of multiple amorphous phases. Furthermore, based on tmDSC data where  $T_g$  values are shifted towards both NIC-rich CASs and VAL-rich CASs, this presence of moisture further facilitates the occurrence of AAPS. This recrystallisation of NIC could be attributed to homogeneous nucleation, as it occurs in the absence of any external interference such as impurity or on the walls of the glass, i.e. not surface driven; all of the recrystallisation occurs within the powdered sample itself (see PLM images).

Homogeneous nucleation can be driven by moisture, where water molecules could aggregate on the surface of the powder. These water molecules are in constant random motion i.e. diffusion. Potentially this adsorption of water disrupts the VAL-NIC bonds by overcoming the critical energy barrier between the VAL and NIC hydrogen bonds, facilitating the migration of NIC molecules. This disruption of bonding in the heterosynthons essentially leads to phase separation and the NIC molecules migrate through the system due to their increased molecular mobility, thus clustering together and nucleate to crystalline form. Only NIC recrystallisation has been detected across all the CASs. Given that these systems had no pre-existing crystals and NIC crystallisation was detected over time, this would suggest that the NIC undergoes homogeneous nucleation to form and grow into crystals.

Heterogeneous nucleation occurs in the presence of external factors such a pre-existing crystal or on the surface of a wall, thus requiring much lower activation energy for the formation of a crystal in comparison to homogeneous nucleation, thus making it an extrinsic process. In case study I, the presence of pre-existing VAL crystals in the SC VAL aids the increase in VAL crystals, i.e. recrystallisation is actually heterogeneous here.

Although the Am VAL adsorbed more moisture than SC VAL, it did not show any significant rise in crystallinity by PLM and maintained its amorphous state

throughout the 32 weeks when stored at 85% RH. The SC VAL did show a small increase in crystallinity by PLM, but no other changes were observed in the overall crystallinity. This suggests that the amorphous form of VAL maintains its amorphosity at high humidity for 32 weeks.

Despite the differences in moisture adsorption, bulk crystallinity, and multiple amorphous phases, all of the binary formulations have demonstrated a gradual increase in crystallinity when stored at 85% RH for a period of 32 weeks. It is important to note that rate of increase in crystallinity varies significantly for the MA and SD CASs when compared to the SL and BM CASs. This variability can be attributed to the different modes of preparation as well as the inhomogeneity within the CASs. When compared together, it does appear that the SL CAS is the most ideal system in terms of overall crystallinity in the system as well as being the one most resistant to crystallinity under high humidity.

## 6.5. Conclusion of Chapter 6

The aim of this chapter is to study the long-term stability against recrystallisation and phase separation of the valsartan and nicotinamide CASs with the optimal composition, prepared by the different processing techniques prepared by four different preparation techniques, namely ball milling, melt amorphisation, spray drying and slurry mixing, by storing them under a range of humidity conditions

It can be inferred that equimolar CASs prepared by different methods, may have different stability against recrystallisation of NIC whilst VAL remains amorphous when stored at a relative humidity of 85%. The presence of moisture disrupts the bonding interaction between the two components causing them to undergo amorphous-amorphous phase separation and ultimately recrystallise, thereby becoming inhomogeneous. This potentially leads to the formation of an amorphous hydrate and have a detrimental effect on the solubility of these systems. The SL CAS is the most stable against recrystallisation and MA and SD CASs being the least. It has been observed that time and humidity allow amorphous-amorphous phase separation in the CASs. The amount of recrystallisation is dependent on the processing route, with the SD CAS demonstrating the highest crystallinity and the SL CAS being lowest after 32 weeks of ageing at 85% RH, possibly due to the presence of trapped moisture in the SD CAS when it was freshly prepared (see [Chapter 5](#) results). Solubility of these systems after 4 weeks of storage in 85% RH drops to the same absolute value for all the systems but retains their solubility values at 25%

RH, indicating that the equilibrium solubilities of co-amorphous systems are independent of recrystallisation tendencies but dependent on the systems' resistance to phase separation when stored at high humidity. However, solubility is unaffected at a lower humidity during that same time, suggesting this performance limiting effect is independent of processing route.

Systems stored at relative humidity of 25% essentially stays amorphous by pXRD for 32 weeks, but some phase separation occurs regardless of the preparation techniques, suggesting that phase separation is an ultimate outcome regardless of how the systems were prepared or stored and they would recrystallise eventually and be detectable by pXRD at a time longer than it took for the system stored at 85% RH. At 25% RH, the MA CAS demonstrated the highest recrystallisation followed by the BM CAS and the solvent-based CASs barely showed any recrystallisation, possibly due to inhomogeneous mixing in the first two techniques.

The drug on its own, did not have exhibited any significant change in its crystallinity after the same ageing process, but it still demonstrated poor aqueous solubility, regardless of ageing compared to the CASs; which further supports the claim that co-amorphisation enhances the solubility of an otherwise poorly soluble drug.

Time and moisture disrupt VAL and NIC secondary bonds leading to amorphous phase separation (including the potential formation of some amorphous hydrate) which allows the homogenous nucleation of crystalline NIC, thus isolating the VAL and reducing its solubility, but still maintaining a higher solubility than that of VAL in both its forms. This work gives a comprehensive understanding of varying moisture conditions impact the performance and stability of the differently processed binary formulations, thereby meeting the original aim of this chapter.

## Chapter 7. Final Conclusion and Future Work

### 7.1. Conclusion

The aim of this work was to prepare co-amorphous formulations of valsartan and nicotinamide by different preparation techniques and test their performances and long-term stability against recrystallisation under high humidity as well as testing their stability during that time under dry conditions (10).

Chapter 4 explored the compositional space of the co-amorphous systems prepared by melt amorphisation. The properties of co-amorphous systems of varying molar compositions of VAL and NIC (3.50: to 1:2) have been synthesised via the thermodynamic disarrangement method. The highly crystalline NIC was not amorphised when it was present in excess ( $>1:2$ ), thus providing an upper limit of amorphisation. The CASs from 2:1 to 1:2 VAL:NIC were X-ray amorphous. It was observed that the equimolar system had the least retained crystallinity by pXRD and PLM, and an excess of either of the components leads to increased moisture adsorption and retained crystallinity increased, both of which can potentially affect long-term stability. tmDSC data of the as-prepared CASs detected the presence of amorphous-amorphous phase separation and even recrystallisation randomly across the compositional space. It was also observed that the plasticisation effects of water (detected by TGA) affect NIC more strongly than VAL due to its polar nature, which lowers the  $T_g$  values of the as-prepared CASs far more significantly in comparison to VAL. A second heating of the now anhydrous CASs confirmed that the experimental  $T_g$ s are close to the expected values from the nominal compositions. VAL solubility was shown to be enhanced for CASs from 2:1 to 1:2 VAL:NIC however, the equimolar system demonstrated the best combination of VAL solubility and recrystallisation resistance of all the CASs when stored in an environment of relative humidity of 85% (since the amorphous state was preserved by pXRD for 4 weeks whereas systems with higher NIC content recrystallised within a week).

Chapter 5 explored different ways of preparing equimolar co-amorphous systems of valsartan and nicotinamide namely melt amorphisation (MA), ball milling (BM), spray drying (SD) and slurry mixing (SL), based on the findings of Chapter 4. Characterisation techniques such as PLM, tmDSC, FT-IR and Raman spectroscopy suggest all the CASs are essentially amorphous with low levels of crystallinity by pXRD and PLM. The two

components are bonded together by hydrogen bonding and  $\pi$ - $\pi$  interaction, thus forming a single homogeneous system and how the two components interact with each other, depends on the mode of preparation. The BM and MA CASs demonstrated inhomogeneity despite having amorphous halos in their pXRD data, suggesting that the two components can exist in amorphous forms without being bonded to each other. The PDF pattern of the SD CAS does not match the other CASs or both forms of VAL, suggesting that the SD CAS has a different structural framework to the rest of the binary systems and the pure components, potentially due to molecules of water or ethanol or both being trapped in the SD CAS. These results confirm that preparation modes affect the mixing and structural packing of the co-amorphous systems. The results also disprove the theoretical idea of a three-molecule heterosynthon due to the lack of any PDF peaks that correspond to this heterosynthon.

The amorphous form of VAL demonstrated the same solubility as the semi-crystalline VAL and showed only a slight improvement in the dissolution rate. This suggests that amorphisation of the drug alone does not enhance its performance. The co-amorphisation techniques however do enhance the aqueous solubility and dissolution. The CASs prepared by solvent evaporation such as spray drying and slurry mixing give the best performance in terms of equilibrium solubility and dissolution improvement and with the little to no crystallinity when freshly prepared, potentially due to ideal mixing in the systems that is difficult to achieve in thermal and mechanochemical methods. Amongst the two solvent evaporated CASs, it can be inferred that slurry mixing is a favourable approach to preparing a CAS as it requires a lesser quantity of solvent to produce a co-amorphous system.

Chapter 6 investigated the long-term stability and solubility of the co-amorphous systems of VAL and NIC prepared by the four different preparation techniques that were explored in Chapter 5, (namely BM, MA, SD, and SL along with the amorphous and semi-crystalline form of VAL). Samples were tested by storing them at relative humidities of 25% and 85% RH at ambient temperature conditions for up to 32 weeks. It was observed that some temporal amorphous-amorphous phase separation (AAPS) occurs in the CASs under dry conditions but that time plus moisture facilitates phase separation as well as recrystallisation. The amount of recrystallisation when stored at 85% RH is dependent on the processing route, with spray drying being highest and slurry mixing being lowest because of inhomogeneous mixing in the thermal technique compared to the solvent technique. It was observed that amorphous VAL alone

maintained its amorphous state at both dry and humid conditions for 32 weeks but the semi-crystalline form of the API demonstrated a slight increase in crystallinity, and it does so in the presence of pre-existing crystal nuclei, aided by the presence of high moisture content, that disrupts the secondary bonds between the VAL molecules. This suggests that recrystallisation in VAL occurs by heterogeneous nucleation.

The aqueous solubility of all the CASs drops to the same absolute level after 4 weeks of ageing in 85% RH, despite their differences in recrystallisation. This performance limiting effect appears to be independent of processing route. The explanation for this may be that time and moisture disrupt secondary bonds between VAL and NIC leading to amorphous phase separation or AAPS (including the potential for formation of some amorphous hydrate) which in turn permits the homogenous nucleation of crystalline NIC, thereby isolating the VAL and reducing its solubility (but still remaining above that of pure VAL). At 25% RH, all the CASs undergo phase separation, evidenced by tmDSC, FT-IR and Raman analysis, with only a very slight increase in crystallinity of the systems evidenced by PLM. Hence, it can be concluded that the co-amorphous systems of VAL and NIC undergo amorphous-amorphous phase separation at both low and high humidities, with the systems recrystallising under high humidities proceeding by the recrystallisation of only NIC whilst VAL maintains its amorphous state under both the conditions.

## 7.2. Final Comments

In summary, this thesis demonstrates that the co-amorphisation of valsartan and nicotinamide enhances the aqueous solubility and dissolution of VAL, thus proving that this method is useful in improving drug delivery systems prepared with BCS Class II drugs. This body of work highlights three important findings, namely

1. The optimum ratio for a stable CAS is equimolar so that all molecules of both components are involved in binding and none are left isolated.
2. Solvent mixing is better than mechanical or thermal mixing approaches for making a homogenous, single-phase CAS, with slurry mixed CAS being the most resilient against recrystallisation.
3. The stability and solubility of a CAS appear to be limited by amorphous-amorphous phase separation and not recrystallisation (although both are enhanced in the presence of moisture where the water molecules disrupt the hydrogen bonding between the components).

### 7.3. Future Work

The work in this thesis can be developed into several extensions, listed below.

#### 7.3.1. Characterisation and modelling of equimolar co-amorphous form products

The co-amorphous form products of valsartan and nicotinamide can be further examined by different characterisation techniques to understand the structure of the binary systems prepared by the four different techniques as well as how they interact and potentially phase separate beyond hydrogen bonding interactions in equimolar systems, thereby addressing the third comment mentioned in section 7.2. Transmission electron microscopy (TEM) is high-resolution imaging technique which can shed new light to the internal structure of the systems, and coupled with electron diffraction, structural differences in the systems prepared in different manners can be identified (98). Terahertz spectroscopy is sensitive to amorphous materials compared to the crystalline counterparts, thus its implementation, coupled with TEM, would be useful for monitoring the physical stability of the co-amorphous systems by detecting low-levels of crystallinity in the early stages as well as detecting hydrates when stored at varying humidity conditions (280). Solid-state nuclear magnetic resonance (ssNMR) can investigate additional secondary interactions such as  $\pi$ - $\pi$  interactions that can occur in systems with aromatic groups and observe how that affect their long-term stability against high humidity and time. ssNMR, when coupled with cross polarisation and magic angle spinning (CPMAS), can quantify crystalline and amorphous phases that can occur in the systems when subjected to varying humidity conditions (281). Dynamic vapour sorption can be implemented to explore the extent of hygroscopicity of the systems by subjecting them to extreme humidity and temperature within a specific period of time, which ultimately assesses their stability against phase separation and recrystallisation (12).

#### 7.3.2. Preparation multiple equimolar co-amorphous form products by slurry mixing with varying solvents

In this thesis, only one organic solvent has been implemented to prepare co-amorphous systems of valsartan and nicotinamide. By using the slurry mixing technique, different solvents such as acetone, isopropanol, ethyl acetate, dichloromethane, dimethyl

sulfoxide and acetonitrile individually as well as in solvent mixtures, can be used to obtain equimolar co-amorphous mixtures of the same components and investigate how varying solvents affect the structures, performance and long-term stability against recrystallisation, as it is the ideal method of preparing CASs with the optimal ratio, as addressed by the first and the second comments in section 7.2. This project can be coupled with computer modelling where DFT can be implemented to model structures in the presence of these solvent(s) molecules in heterosynthons and can simulate spectroscopic data and structural information which would provide insight into the intermolecular interactions at a molecular level. Such a comparative study would elucidate the co-amorphisation ability of different kinds of solvents and how this affects the resistance to phase separation and recrystallisation of the binary drug system.

### 7.3.3. Assessing the efficacy and stability of ternary systems

In this thesis, slurry mixing or rotary evaporation has been determined as the most optimal method of preparation of co-amorphous systems as it consumes the least solvent, produces a homogeneous system with no retained crystallinity, enhanced solubility and dissolution of valsartan and is the most resistant to recrystallisation. However, all of these features can be improved by the incorporation of a suitable third component, thereby producing a ternary system using the optimal method of solvent evaporation technique, as presented in the second comment in section 7.2. A ternary system can potentially enhance solubility and dissolution and improve stability against AAPS and recrystallisation, thereby addressing the limitations of CASs, as mentioned in the third comment of section 7.2. A CAS utilises the optimal equimolar ratio, as stated in first comment in section 7.2, whilst the incorporation of a third component in a ternary system may reduce its drug loading capacity in the final dosage form. However, such an issue can be addressed by increasing the drug load or decreasing the third component quantity in a manner that does not adversely affect the AAPS and recrystallisation and by carefully selecting the third component that forms strong secondary interactions with the other components of the drug delivery system. The third component could be a low hygroscopic, enteric polymer like hydroxypropyl methylcellulose acetate succinate (HPMCAS), forming a drug-excipient-polymer system, or a drug-excipient-surfactant system with sucrose laurate as the third component (282). Both of these co-formers are plant-based components that are generally regarded as safe (GRAS) and could potentially be suitable components to combine with valsartan and nicotinamide as the third co-former

to prepare two different ternary systems. These could then be evaluated for the difference in solubility, dissolution, and stability against phase separation and recrystallisation by analysing them with different characterisation techniques. Such a study would provide a detailed comparative analysis between a binary system of valsartan and nicotinamide as well as with two ternary systems with two different kinds of ternary co-formers.

Ultimately, this thesis has demonstrated that co-amorphisation enhances the performance of BCS Class II drugs and how preparation methods may affect long-term stability of co-amorphous systems against AAPS and recrystallisation. Importantly, this stability is limited when stored under high humidity. Thus, co-amorphisation is a favourable method of preparing drug delivery systems with enhanced performance, however, it is important to carry out research on the long-term stability of these systems with different components or additional components to understand how moisture plus time affect stability and performance, so that drug delivery systems with the best efficacy and resistance to phase separation and transformation can be brought to the global market.

## Appendix A. Data Acquisition and Analysis for Chapter 4

**Table A.1:** Additional endothermic events of glass transition ( $T_g$ ) corresponding to VAL and melting ( $T_m$ ) corresponding to VAL and NIC across systems in the first heating cycle.

| <u>Ratio (VAL:NIC)</u> | <u><math>T_g</math></u> | <u><math>T_m</math></u> |
|------------------------|-------------------------|-------------------------|
| 1:2                    | 74.4±1.8                | 95.5±0.9                |
| 1:1.75                 | -                       | -                       |
| 1:1.50                 | -                       | 108.2±6.5               |
| 1:1.25                 | -                       | 103.0±5.4               |
| 1:1                    | 66.7±1.9                | 108.2±1.0               |
| 1.25:1                 | -                       | -                       |
| 1.50:1                 | 77.8±0.6                | 121.3±1.9               |
| 1.75:1                 | 80.5±2.1                | 125.0±3.5               |
| 2:1                    | -                       | -                       |
| 2.25:1                 | -                       | 125.4±2.2               |
| 2.50:1                 | -                       | -                       |
| 2.75:1                 | -                       | -                       |
| 3:1                    | -                       | -                       |
| 3.25:1                 | -                       | 104.4±0.5               |
| 3.50:1                 | -                       | -                       |

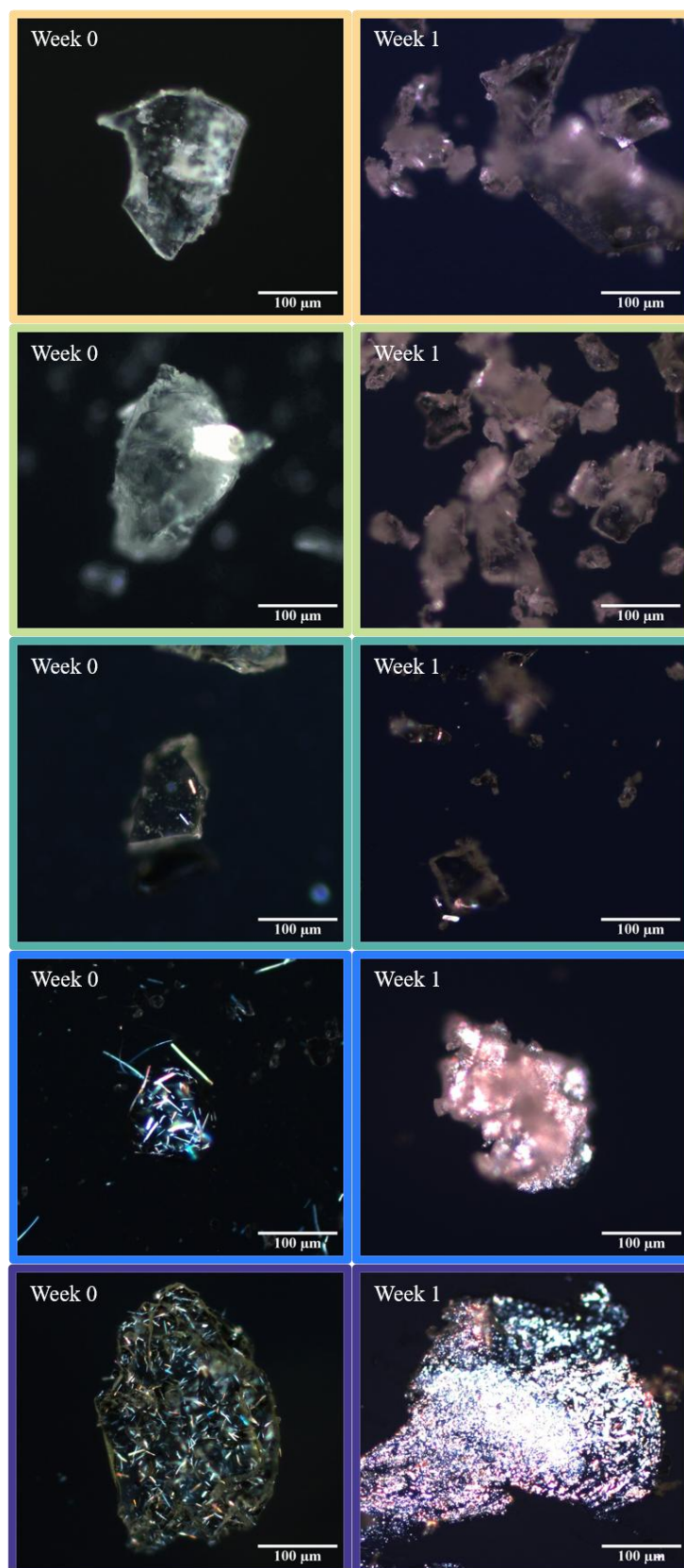


**Figure A.1:** PLM images of MA CASs of VAL:NIC in the ratios 2:1, 1.5:1, 1:1, 1.5:1 and 1:2, from top to bottom (bordered in orange-yellow, light green, teal, blue and violet respectively) at weeks 0 and 1 at 85% RH. Crystallinity was evaluated by analysing 10 images per sample, at a given time.

## Appendix B. Data Acquisition and Analysis for Chapter 5

**Table B.1:** Endothermic events of glass transition ( $T_g$ ) and melting ( $T_m$ ) across co-amorphous systems prepared by different methods in the first heating cycle.

| <u>Ratio (VAL:NIC)</u> | <u><math>T_g</math></u> | <u><math>T_m</math></u> |
|------------------------|-------------------------|-------------------------|
| Ball Milled CAS        | 43.6±0.9                | 119.4±1.1               |
| Melt Amorphised CAS    | 32.0±0.1,<br>66.7±1.9   | 108.2±1.0               |
| Spray Dried CAS        | 45.9±1.8                | -                       |
| Slurry Mixed CAS       | 35.1±2.3                | -                       |

### HPLC related information

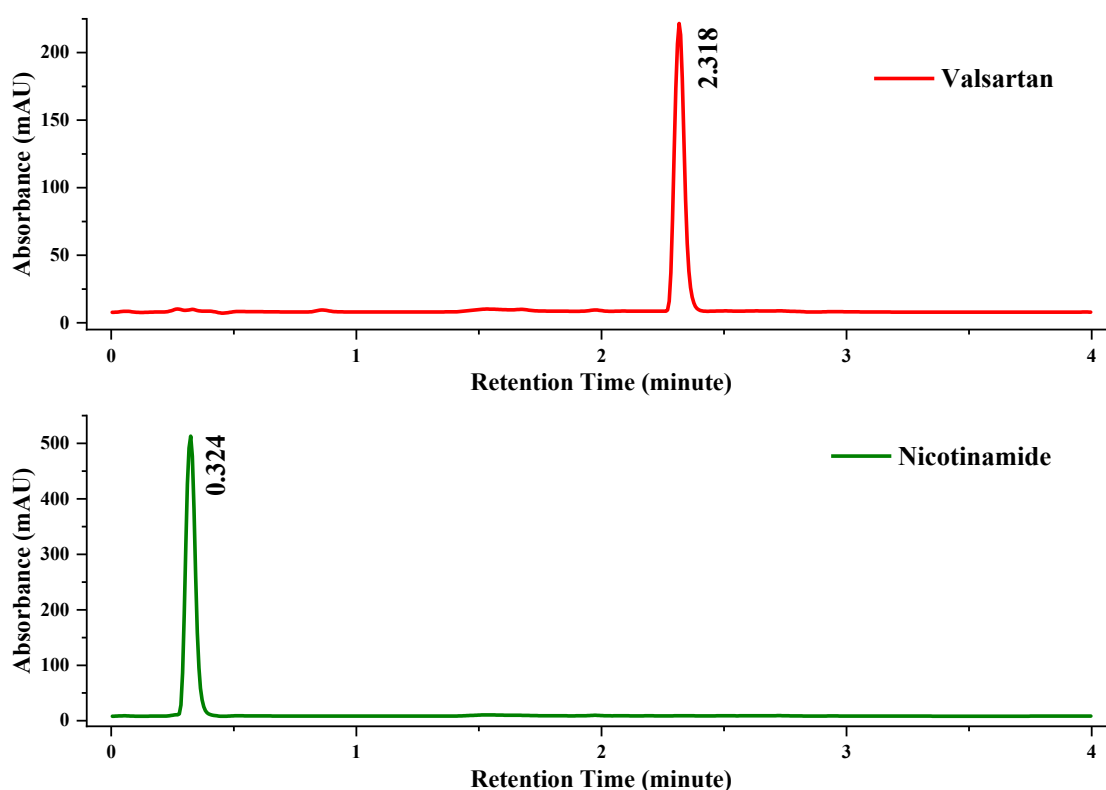


**Figure B.1:** Chromatograms of valsartan (red) and nicotinamide (green).

- Limit of Quantification for VAL and NIC are  $6.6 \mu\text{g mL}^{-1}$  and  $2.4 \mu\text{g mL}^{-1}$ , respectively. The limit of detection (LOD) was not determined, as establishing absolute detection boundaries was unnecessary for the purpose of this

quantification process. Instead, the analytical method was validated within a defined working range from 2.4  $\mu\text{g mL}^{-1}$  to 577.0  $\mu\text{g mL}^{-1}$ . This specific range was selected because it encompasses the exact concentrations expected to be prepared and analysed within the experimental mixtures.

- Chromatographic separation of the target compounds yielded sharp, well-defined peaks with a high degree of separation. Nicotinamide eluted first with a retention time of 0.32 min (peak width of 0.04 min), followed by valsartan at a retention time of 2.32 min (peak width of 0.05 min). The method demonstrated an exceptional resolution of 44.71, far exceeding the regulatory requirement of 1.5.
- The specificity of the developed HPLC method was evaluated by monitoring the eluent using a Diode Array Detector across the UV spectrum. Peak purity analysis confirmed the total absence of co-eluting impurities or degradation products within both the nicotinamide and valsartan peak profiles, establishing the high accuracy and precision of the method.

## Appendix C. Data Acquisition and Analysis for Chapter 6

### Case Study I: Valsartan (Semi-crystalline and amorphous)



**Figure C.1:** PLM images of SC VAL and Am VAL (bordered in magenta and maroon respectively) at weeks 0 and 32 at 25% RH. Crystallinity was evaluated by analysing 10 images per sample, at a given time.

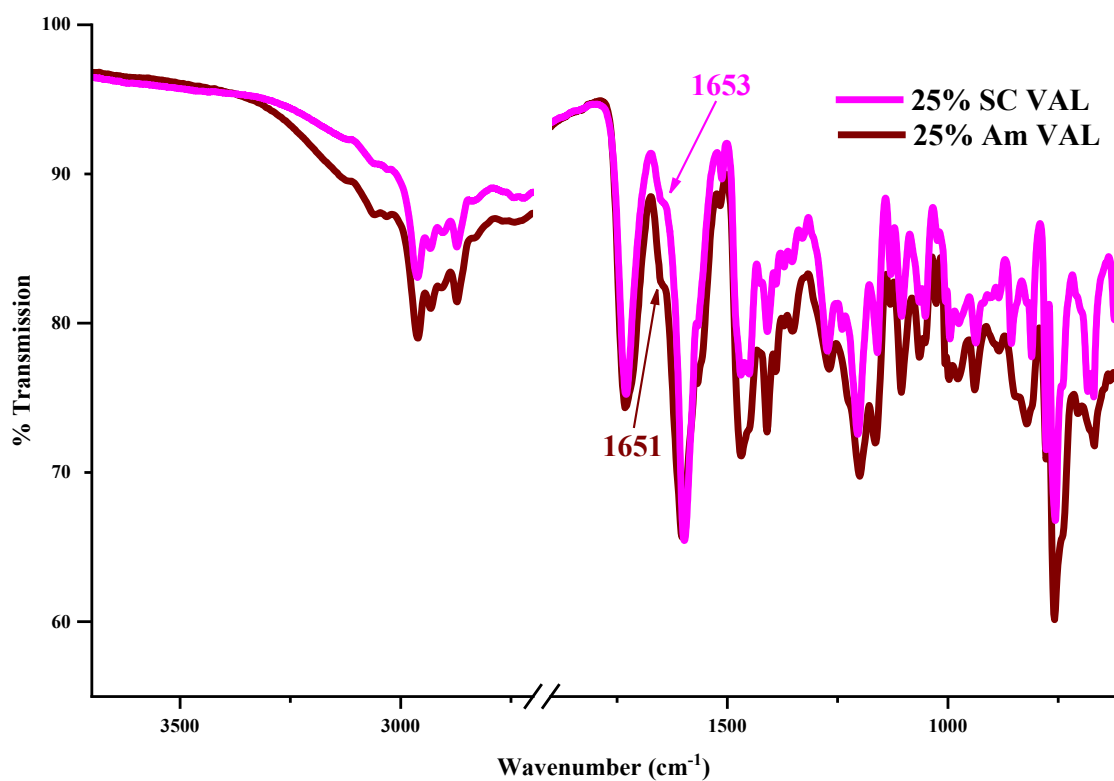


**Figure C.2:** FT-IR spectra of SC and Am VAL at 25% RH at week 32.

**Table C.1:** Experimental Values of FT-IR spectra for SC VAL and Am VAL at 25% RH at week 0 and week 32 along with the shifts ( $\Delta$ ) in values of VAL prior to ageing (fresh).

| <b><u>Functional Groups</u></b>     | <b><u>Pure VAL</u></b> |                       | <b><u>Am VAL</u></b> |                       |
|-------------------------------------|------------------------|-----------------------|----------------------|-----------------------|
|                                     | <b><u>Week 0</u></b>   | <b><u>Week 32</u></b> | <b><u>Week 0</u></b> | <b><u>Week 32</u></b> |
| (O-H) stretching                    | 3116                   | 3116                  | 3116                 | 3114 ( $\Delta=-2$ )  |
| $\nu(\text{NH})_{\text{tetrazole}}$ | 3455 (poor signal)     | 3455 (poor signal)    | 3455 (poor signal)   | 3455 (poor signal)    |
| Acid (C=O) stretching               | 1729                   | 1730 ( $\Delta=+1$ )  | 1729                 | 1732 ( $\Delta=+3$ )  |
| Amide (C=O) stretching              | 1597                   | 1598 ( $\Delta=+1$ )  | 1598                 | 1603 ( $\Delta=+5$ )  |

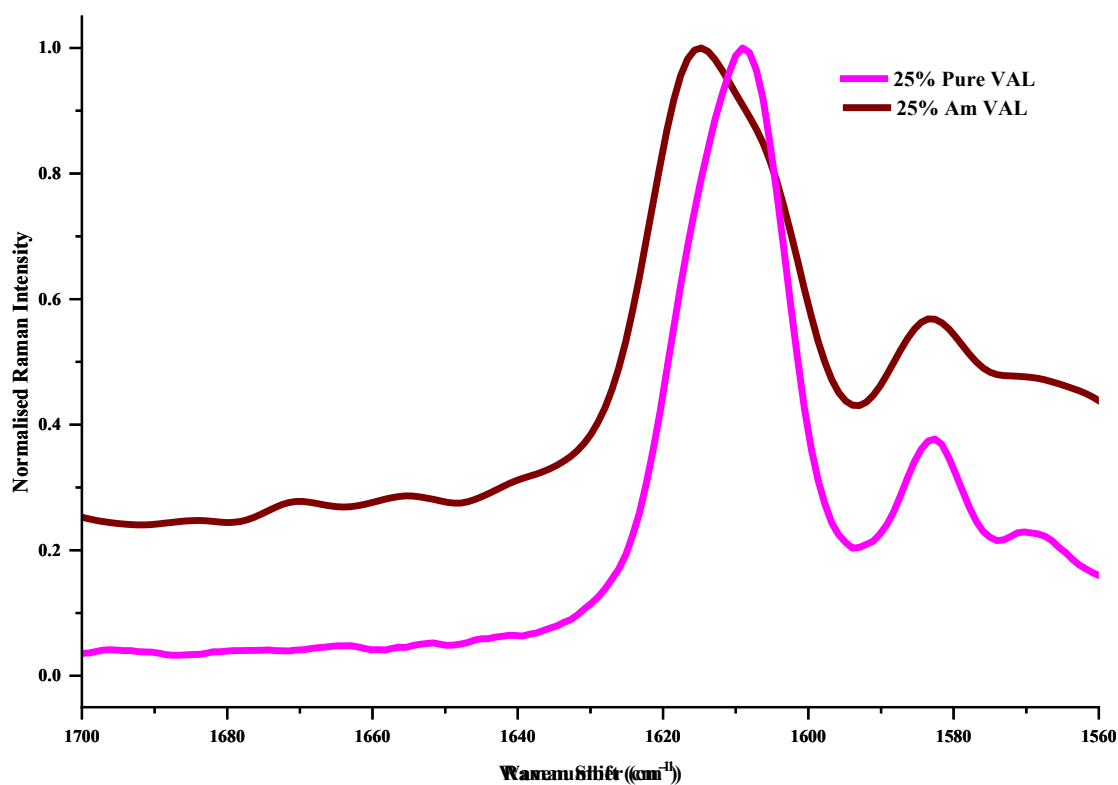


**Figure C.3:** Raman spectra of SC and Am VAL at 25% RH at week 32.

**Table C.2:** Experimental Values of Raman spectra for SC VAL and Am VAL at 25% RH at week 32 along with the shifts ( $\Delta$ ) in values of VAL prior to ageing (fresh).

| <u>Functional Groups</u> | <u>Pure VAL</u> |                      | <u>Am VAL</u> |                      |
|--------------------------|-----------------|----------------------|---------------|----------------------|
|                          | <u>Week 0</u>   | <u>Week 32</u>       | <u>Week 0</u> | <u>Week 32</u>       |
| C=O (amide) <sub>s</sub> | 1608            | 1609 ( $\Delta=+1$ ) | 1609          | 1615 ( $\Delta=+6$ ) |
| N-H <sub>b</sub>         | 1564            | 1564                 | 1564          | 1564                 |
| C=C <sub>s</sub>         | 1583            | 1583                 | 1583          | 1583                 |

## Case Study II: Co-amorphous systems of VAL and NIC

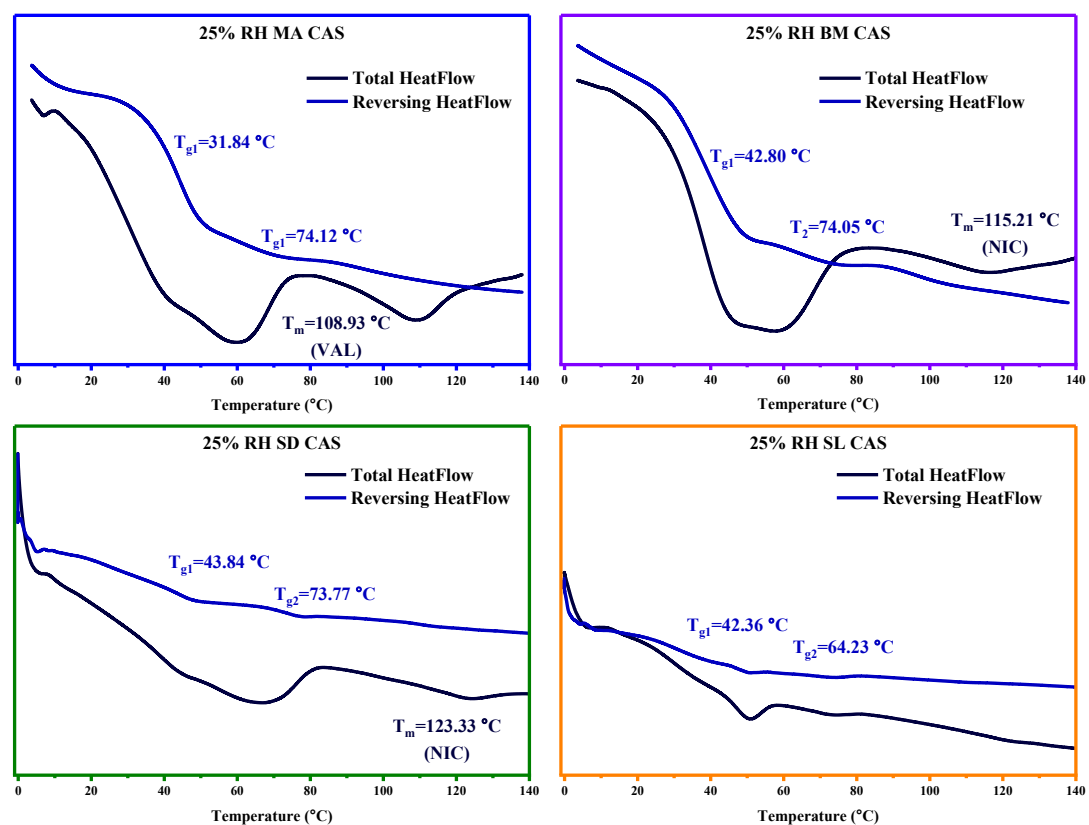


**Figure C.4:** tmDSC plot with total and reversing heat flow signals of MA, BM, SL and SD CASs at week 32 for 25% RH.

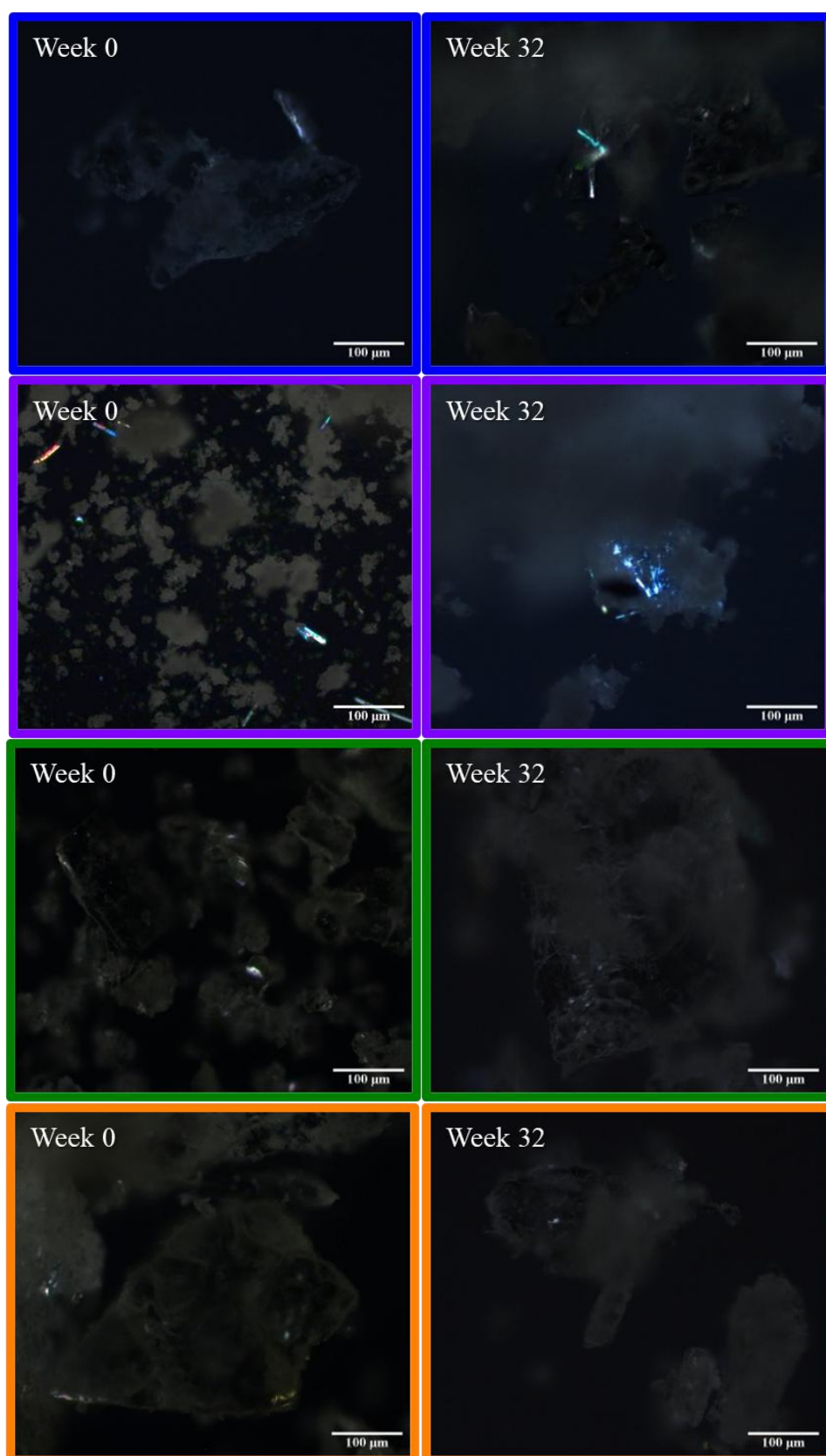


**Figure C.5:** PLM images of MA, BM, SD, and SL CASs (bordered in blue, purple, green and orange respectively) at weeks 0 and 32 at 25% RH. Crystallinity was evaluated by analysing 10 images per sample, at a given time.

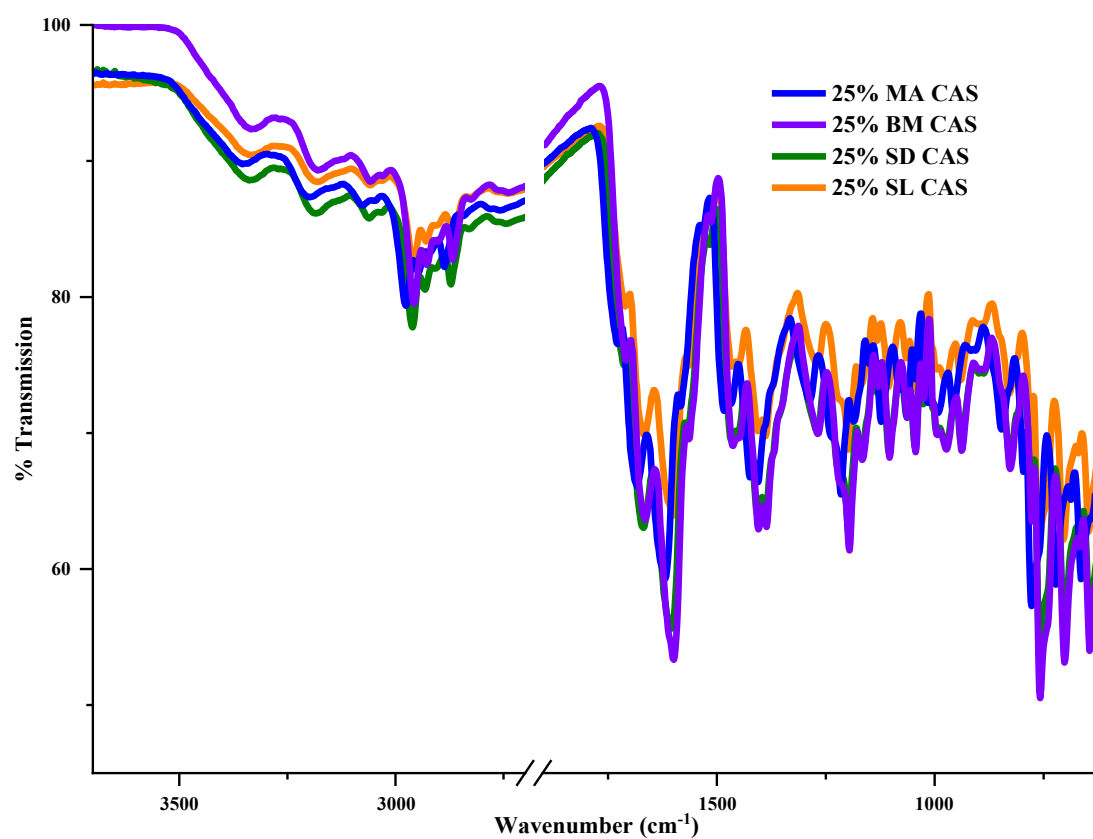


**Figure C.6:** FT-IR spectra of MA, BM, SD, and SL CASs at 25% RH at week 32.

**Table C.3:** Experimental Values of FT-IR spectra for MA, BM, SD, and SL CASs at 25% RH at week 32 along with the shifts ( $\Delta$ ) in values of CASs prior to ageing (fresh).

| <b><u>Functional Groups</u></b>     | <b><u>Frequency (cm<sup>-1</sup>)</u></b>   |                                  |                                  |                                  |
|-------------------------------------|---------------------------------------------|----------------------------------|----------------------------------|----------------------------------|
|                                     | <b><u>MA CAS</u></b>                        | <b><u>BM CAS</u></b>             | <b><u>SD CAS</u></b>             | <b><u>SL CAS</u></b>             |
| (O-H) stretching                    | 3205 ( $\Delta=+31$ )                       | 3184 ( $\Delta=+1$ )             | 3186 ( $\Delta=+4$ )             | 3184 ( $\Delta=+3$ )             |
| $\nu(\text{NH})_{\text{tetrazole}}$ | -                                           | -                                | -                                | -                                |
| Acid (C=O) stretching               | 1730 ( $\Delta=+13$ )                       | 1715 ( $\Delta=+2$ )             | 1715 ( $\Delta=+2$ )             | 1713 ( $\Delta=+1$ )             |
| Amide (C=O) stretching              | 1620<br>(Shoulder at 1631) ( $\Delta=-17$ ) | 1603<br>(Shoulder at 1616)       | 1603<br>(Shoulder at 1614)       | 1603<br>(Shoulder at 1622)       |
| Unreacted (N-H) as. stretching      | 3356 ( $\Delta=-2$ )                        | 3333 ( $\Delta=-3$ )             | 3338 ( $\Delta=+1$ )             | 3336 ( $\Delta=-20$ )            |
| (N-H) as. stretching                | 3205 ( $\Delta=+31$ )                       | 3184 ( $\Delta=1$ )              | 3186 ( $\Delta=+4$ )             | 3184 ( $\Delta=+3$ )             |
| (N-H) stretching                    | 3078 ( $\Delta=+18$ )                       | 3061                             | 3061 ( $\Delta=-1$ )             | 3061 ( $\Delta=-1$ )             |
| (C=O) stretching                    | 1686 ( $\Delta=+33$ )                       | 1670 ( $\Delta=+2$ )             | 1670 ( $\Delta=+2$ )             | 1670 ( $\Delta=+5$ )             |
| (N-H) bending                       | 1620, 1583<br>( $\Delta=+17, +16$ )         | 1603, 1566<br>( $\Delta=0, -1$ ) | 1603, 1566<br>( $\Delta=0, -1$ ) | 1603, 1568<br>( $\Delta=0, +1$ ) |

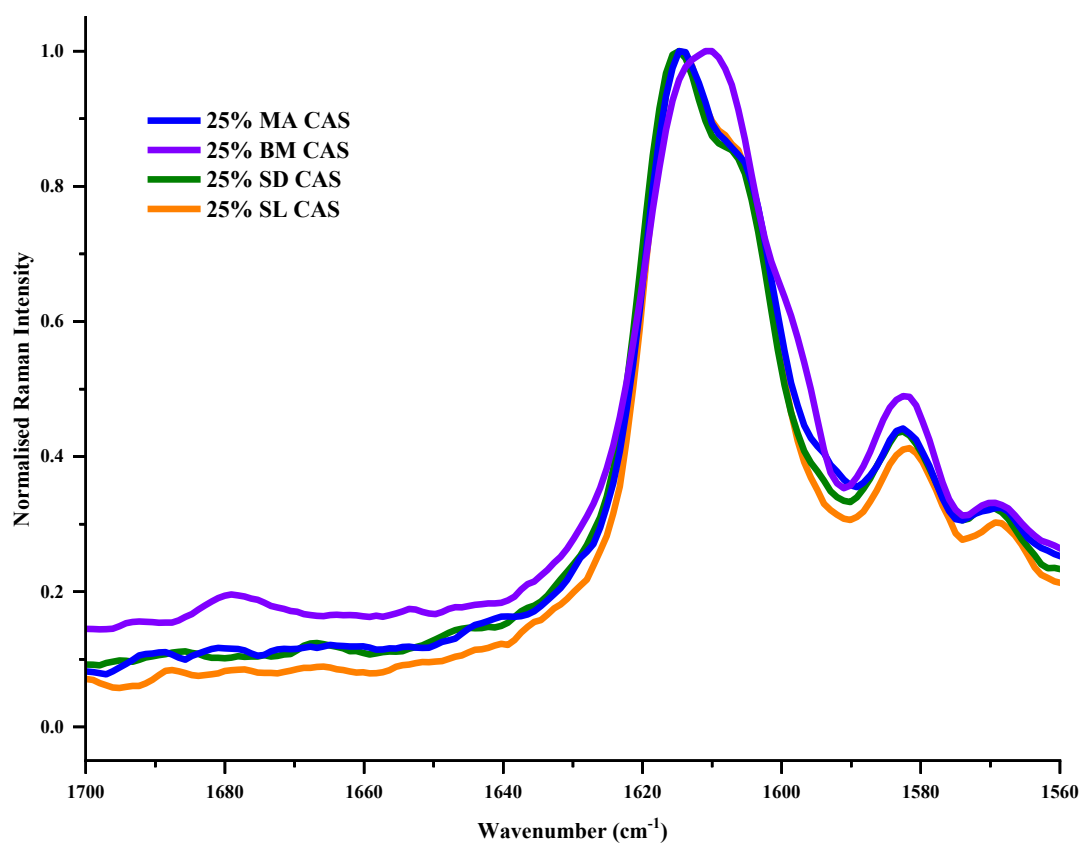


**Figure C.7:** Raman spectra of MA, BM, SD, and SL CASs at 25% RH at week 32.

**Table C.4:** Experimental Values of Raman spectra for MA, BM, SD, and SL CASs at 25% RH at week 32 along with the shifts ( $\Delta$ ) in values of CASs prior to ageing (fresh).

| <u>Components</u> | <u>Functional Groups</u> | <u>MA CAS</u>                                | <u>BM CAS</u>                                 | <u>SD CAS</u>                            | <u>SL CAS</u>                                |
|-------------------|--------------------------|----------------------------------------------|-----------------------------------------------|------------------------------------------|----------------------------------------------|
| VAL               | C=O (amide) <sub>s</sub> | 1613, ( $\Delta = -2$ ), shoulder at 1605    | 1610 ( $\Delta = -1$ ), shoulder at 1600      | 1615, shoulder at 1605                   | 1615, ( $\Delta = +1$ ), shoulder at 1607    |
|                   | N-H <sub>b</sub>         | 1568 ( $\Delta = -2$ )                       | 1569                                          | 1570                                     | 1569                                         |
|                   | C=C <sub>s</sub>         | 1568 ( $\Delta = -2$ )                       | 1569                                          | 1570                                     | 1569                                         |
| NIC               | C=O (amide) <sub>s</sub> | Goes undetected                              | 1679                                          | Goes undetected                          | Goes undetected                              |
|                   | N-H <sub>b</sub>         | UD, 1605 (merged with VAL) ( $\Delta = -1$ ) | UD, 1600 (merged with VAL) ( $\Delta = -11$ ) | 1605 (merged with VAL) ( $\Delta = -3$ ) | UD, 1607 (merged with VAL) ( $\Delta = +1$ ) |
|                   | C=C <sub>s</sub>         | 1568 ( $\Delta = -2$ )                       | 1569                                          | 1570                                     | 1569                                         |

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