Molecular Mechanisms Influencing the Performance of Amorphous Formulations for Poorly Water-Soluble Drugs

KHADIJAH EDUENG
Abstract

Crystallisation is a concern for amorphous formulation because it compromises the solubility-enhancing benefit gained from amorphisation. Traditionally, amorphous formulation had been designed primarily based on trial-and-error approach. The success rate for amorphous formulation is unimpressive, due to a poor understanding of the formulation itself, especially with regard to its crystallisation behaviour. Therefore, this thesis aimed to propose a strategic approach for rational design of amorphous formulations, as opposed to the trial-and-error approach. This can be achieved by understanding what drives the crystallisation of amorphous drug, and when and how the amorphous drug crystallises. The information can guide the selection of drugs, excipients and preparation method to achieve amorphous formulations with favourable features.

In the first part of the thesis, a systematic protocol was proposed to identify mechanisms via which crystallisation takes place when amorphous drug is dissolved. The stabilisation strategy of supersaturation produced upon dissolution of amorphous drug was then recommended depending on the crystallisation mechanisms. A molecular dynamics (MD) simulations was used to understand drug-polymer interaction during supersaturation. It was revealed that hydrogen bond interaction is an important in stabilising supersaturation. The factors affecting glass-forming ability and long-term physical stability such as preparation method and humidity were then highlighted in the second study. A follow-up study was performed to elucidate the potential complications in using a standardised differential scanning calorimetry to classify promiscuous glass formers into any specific glass-forming ability/glass stability class. In the subsequent study, the effect of physical aging and/or crystallisation of amorphous drugs during storage on supersaturation potential was addressed. It was shown that, minor crystallisation of amorphous drug upon storage did not have a significant impact on the supersaturation potential during dissolution. Instead, the crystallisation pathway of the amorphous drug during dissolution plays a more important role in determining the supersaturation behaviour of some drugs. Finally, the impact of (i) drug loading on physical stability, supersaturation, drug/polymer miscibility, and (ii) the physical aging and/or crystallisation upon storage on supersaturation potential of spray-dried solid dispersions with HPMC-AS were discussed in the last study. It was observed that the effect of drug loading on physical stability and supersaturation, and the effect of physical aging and/or crystallisation during storage on supersaturation potential is highly drug-dependent. Similarly, the stabilisation effect of HPMC-AS varied across model drugs, drug loadings and crystallisation pathways (i.e. in solid or during dissolution). The Flory-Huggins interaction parameter calculated using MD simulations revealed good miscibility between the drugs and HPMC-AS at drug loadings investigated. In the presence of water molecules, various structural organizations of the drugs and HPMC-AS complexes were observed. Taken together, this thesis provides an improved understanding of crystallisation behaviour of amorphous formulations, which is useful to guide a rational design of amorphous formulations.

Keywords: Amorphous formulation, crystallisation, supersaturation, glass-forming ability, physical stability, glass stability, spray-dried solid dispersion, dissolution, promiscuous glass former, poorly-soluble drug, solid-to-solid, solution-mediated, particle-associated

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To my family and in loving memory of my beloved parents
“If my mind can conceive it, and my heart can believe it—then I can achieve it”

Jesse Jackson
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Abbreviations

ASD  Amorphous solid dispersion
AUC  Area under the curve
C_{max,app}  Apparent maximum concentration
DSC  Differential scanning calorimetry
GF   Glass former
GFA  Glass-forming ability
GS   Glass stability
HBA  Number of hydrogen bond acceptor
HBD  Number of hydrogen bond donor
k    Crystallisation rate constant
logP Octanol-water partition coefficient
MQ   Melt-quenched
MW   Molecular weight
nGF  Non-glass former
PLM  Polarised light microscopy
PSA  Polar surface area
PXRD Powder X-ray diffraction
R_{crit} Critical cooling rate
RH   Relative humidity
RMSD Root mean square deviation
RMSF Root mean square fluctuation
RS   Relative saturation
RotB Number of rotatable bond
SD   Spray-dried
SEM  Scanning electron microscopy
T_c  Crystallisation temperature
T_g  Glass transition temperature
T_m  Melting temperature
T_{rg} Reduced glass transition temperature
\Delta H_f Heat of fusion
\Delta S_f Entropy of fusion
\chi  Flory-Huggins interaction parameter
Introduction

This section provides background relevant to the thesis and knowledge gaps in the field.

Applications of amorphous formulations

Drug development is highly dependent on the optimisation of the physico-chemical properties of the drug molecule during an early stage of the process. This is achieved through various synthesis processes\(^1\), research organisation behaviour,\(^2,\) and by understanding the target biology\(^4-6\). These approaches resulted in a trend to discover compounds with molecular features such as increased molecular weight and lipophilicity which lead to limited aqueous solubility\(^7\).

Solubility, together with permeability, are the two most important properties for oral absorption. These two properties are the cornerstones of the Biopharmaceutics Classification System (BCS), which classifies compounds into four different classes\(^8\). The BCS Class II and IV compounds have poor water solubility, with the difference being that Class IV compounds also have limited permeability. Since the oral route is the preferred option for the administration of drug compounds – due to its convenience and good patient compliance – sufficient water solubility of the molecule is important to ensure complete absorption of drug from the gastrointestinal (GI) tract\(^9\). Only dissolved drug molecules can permeate the gastrointestinal epithelia.

Nevertheless, between 40-70% of these new molecules are too poorly soluble to allow complete absorption from the GI tract\(^10\). This attracted interest in researching formulation strategies to overcome the solubility problem\(^11-19\), with amorphous formulation being one of the most widely studied strategies\(^20-22\).

In theory, compounds with solid-state limited solubility would benefit from amorphisation. These compounds often identifiable by their high melting temperature (\(T_m\))\(^23\). During amorphisation, the strong crystal structure would be disrupted leading to a weaker amorphous solid structure with a short-range molecular arrangement. Other potential benefits besides a weakening of the intermolecular bonds within the crystal structure are a decrease in the particle size\(^24,25\), and modifications of the overall lipophilicity and/or hydrophilicity.
Therefore, the applicability of amorphisation as a strategy for solubility enhancement extends beyond solid-state limited compounds. It has been used for solvation-limited compounds, where solubility is limited by their highly lipophilic nature. The applicability of amorphous formulation is evident from the wide distribution of amorphous-based drugs on the market.

Preparation of amorphous formulations

Amorphous formulations can be prepared by different methods. These methods can be classified as solvent-based, temperature-based (fusion), and mechanical-based (activation). In some cases, these methods are used in combination with each other. Solvent-based methods are the most common and include spray-drying, freeze-drying, precipitation, solvent evaporation, supercritical fluid approaches, and different types of electro-spraying. Among these, spray-drying is one of the most widely used and applicable methods in the pharmaceutical industry.

Characterisation and performance evaluation of amorphous formulations

Solid materials can be amorphous, crystalline, polymorphic or pseudopolymorphic, each having distinguishable characteristics. They can be identified using a number of different solid-state characterisation techniques. Not all solid materials are amorphous and methods are required to differentiate between amorphous and other types of solid materials. Very often, these methods are used in tandem for clearer and more conclusive interpretation of the characteristics of the solid materials studied. Some of the most commonly used methods are summarised in Table 1. After characterisation of the solid-state forms, an amorphous formulation is assessed for its physical stability, in vitro and/or in vivo solubility, dissolution, absorption and pharmacokinetic profiles.
Table 1. Solid-state characterisation techniques.

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<td>Scanning electron microscopy</td>
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Advantages of amorphous formulations

In terms of dissolution and solubility, a well-functioning amorphous formulation offers multifaceted benefits. The main benefit comes from the lack of long-range order compared to the corresponding crystalline counterpart. Lack of long-range order decreases the energy barrier imposed by the material during the dissolution process \(^{56,57}\). In vivo, the faster dissolution and higher solubility of the amorphous solid often leads to a higher concentration of free drug available for absorption through the GI tract \(^{58}\), causing a phenomenon called supersaturation \(^{59,60}\). Additionally, the amorphous formulation may form colloidal species upon dissolution, which are more readily available than the undissolved solid form, and thus enhance the dissolution rate even further \(^{61-63}\). Finally, the presence of an excipient may prolong and stabilise the solution in its supersaturated state for a physiologically relevant time, by delaying and/or inhibiting crystallisation and precipitation from the supersaturated solution \(^{64-66}\).

Crystallisation: The key problem of amorphous formulations

Due to its thermodynamic instability, an amorphous compound has the tendency to undergo crystallisation, which negatively affects its storage stability and/or supersaturation potential following dissolution. This imposes a major setback to the amorphous system and its application as a viable formulation for the solubility enhancement of poorly water-soluble drugs. Therefore, the successful implementation of an amorphous formulation is highly dependent on efficient control of the drug crystallisability, starting from its manufacturing and up to its dissolution in vivo upon oral administration.

In general, crystallisation involves nucleation (i.e., formation of nuclei or seed crystals) followed by crystal growth. Crystallisation can take place in
solid-state, solution or during dissolution of amorphous solid (Figure 1). In the solid-state, crystallisation initiates on the solid surface and/or in the bulk of the solid. Crystal growth propagates faster on the surface than in the bulk of amorphous solid and this phenomenon results in the formation of a thin layer of crystal around the relatively slower crystallising bulk 67-69. This is due to higher mobility 70 and lower elastic strain on the surface of the amorphous solid 71, which increases the thermodynamic driving force for crystallisation.

Figure 1. Different pathways of crystallisation involving amorphous solid, in solution and during dissolution of amorphous solid.

In a solution, crystallisation proceeds homogeneously or heterogeneously (Figure 1). Homogenous nucleation takes place in a pure system (without impurities) and stimulated by supersaturation of the bulk solution. The activation for crystallisation requires higher degree of supersaturation. Heterogeneous nucleation, on the other hand, is triggered at a relatively lower supersaturation and initiated on a solid surfaces (e.g. dust, stirrer, vial, drug particles) 72.

During the dissolution of amorphous solid, crystallisation can be induced via two major mechanisms – solid-to-solid and solution-mediated crystallisation 58 (Figure 1). The solid-to-solid crystallisation minimises the degree of supersaturation generated, whereas solution-mediated crystallisation limits the time during which the system is in supersaturated state. In some cases, crystallisation from both pathways can take place simultaneously.

It has been described in the literature that solid-to-solid crystallisation initiates on the surface of the amorphous particle, where its surface molecules are exposed to water. This exposure results in that the surface molecules being plasticised, which in turn lowers the glass transition temperature ($T_g$), in-
creases the molecular mobility, and hence increases the crystallisation tendency \(^{73,74}\). However, based on the Nernst-Brunner dissolution theory \(^{75,76}\) (Figure 2a and Equation 1), it can also be hypothesised that supersaturation can be generated at the diffusion layer (or close to the solid particle-liquid interface) because the amorphous solid has higher solubility than its crystalline counterpart as it dissolves. Since the solubility generated by the dissolution of amorphous solid at the diffusion layer is kinetic in nature, it is inherently unstable. This in turn increases the driving force for crystallisation to achieve thermodynamic stability. Crystallisation via this pathway is known as particle-associated. Based on this hypothesis, the Nernst-Brunner equation can therefore be modified as

\[
\frac{dC}{dt} = \frac{DS}{Vh} (C_s - C_b) \tag{1}
\]

where \(dC/dt\) is the dissolution rate, \(D\) is diffusion coefficient of solute in solution, \(S\) is the surface area of exposed amorphous solid, \(h\) is the thickness of the diffusion layer, \(V\) is the volume of the solution, \(C_s\) is the solubility of the amorphous solid (i.e., concentration of saturated solution of the compound at the surface of the amorphous solid (\(x=0\)) and at the temperature of the experiment), and \(C_b\) is the concentration of solute in the bulk solution (\(x=h\)) at time \(t\). Dissolution theory assumes that the aqueous diffusion layer of thickness \(h\) exists at the surface of a solid undergoing dissolution. There is a major limitation in measuring supersaturation within a diffusion layer of dissolving amorphous solid. Therefore, the latter hypothesis, which is based on Nernst-Brunner dissolution theory has not been previously proposed, studied and discussed in great details.

Another proposed mechanism of crystallisation for amorphous solid during dissolution is mediated from the bulk solution. This mechanism is commonly known as solution-mediated crystallisation. Via this mechanism, the crystallisation is initiated by the formation of supersaturated bulk solution as the amorphous solid dissolves.\(^{58,77,78}\)

The main implications of crystallisation from the amorphous solid are: (i) physical instability (if the crystallisation occurs in its solid form); and (ii) lack of supersaturation or unstable supersaturation (if the crystallisation takes place during dissolution). Depending on the extent of crystallisation, the benefit resulting from solubility enhancement gained from amorphisation will be compromised in both cases (Figure 2b). Therefore, it is of paramount importance to prevent crystallisation from occurring in either or both pathways to preserve the stability of amorphous solid form and maintain a stable supersaturation for a physiologically relevant time.
Glass-forming ability and glass stability

Due to the inherent tendency of amorphous solids to crystallise, many initiatives have been taken to identify the ease at which the crystalline solids transform to the amorphous state and how well they resist crystallisation. These are more commonly described as glass-forming ability (GFA) and glass stability (GS), respectively. GFA and GS provide a qualitative estimation regarding the crystallisation tendency of a compound, which is an indicator of its suitability for formulation as an amorphous dosage form.

Various structural and kinetic theories have been proposed to understand GFA. The critical cooling rate \( R_{\text{crit}} \) is the most commonly used parameter to determine the GFA of materials. This \( R_{\text{crit}} \) is defined as the minimum cooling rate required to vitrify materials. The estimation of \( R_{\text{crit}} \) necessitates the construction of isothermal time-temperature-transformation or continuous cooling curves. The major limitation of this method is that it is laborious, and can therefore not be performed on a large number of samples. In addition, the theoretical calculation of the curves is typically not possible due to the lack of accurate nucleation rate experiments.

Due to these drawbacks, another method, melt-quenching (MQ), was established to measure \( R_{\text{crit}} \) using differential scanning calorimetry (DSC).
This method was later refined to improve its predictive power \cite{85} and is now considered to give an accurate prediction of $R_{\text{crit}}$ of inorganic materials \cite{85}. In melt-quenching, the compounds are subjected to a heat-cool-heat cycle in the DSC at a standard heating and cooling rate. Melt-quenching in the DSC has been used to investigate the GFA and to classify a large number of compounds \cite{86}, but a rapid solvent evaporation method has also been reported to give a reasonable correlation between the GFA/GS classes of compounds \cite{87}.

A compound is classified as Class I, if the melt crystallises during the cooling cycle; Class II, if the compound crystallises upon the second heating; or Class III, if the compound does not crystallise upon cooling and second heating (Figure 3). The same interpretation of the DSC thermogram is used for the GFA/GS classification of compounds prepared by rapid solvent evaporation and spray-drying, except that the GFA/GS classes are assigned based on only one heating run in the DSC.

The findings from the GFA/GS classification studies have attracted a lot of interest but the following information is still lacking and requires more studies: (i) does the GFA/GS classification hold true when spray-drying is used instead of melt-quenching (especially for larger datasets)?; and (ii) what is the predictability and relationship of GFA/GS classification and the long-term storage stability profiles under humid conditions?

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Glass-forming ability/glass stability classification based on thermal behaviour upon a heat-cool-heat cycle in the differential scanning calorimeter. Class I is a non-glass former that crystallises during cooling, Class II is an unstable glass-former that crystallises upon the second heating, and Class III is a stable glass-former which does not crystallise either during cooling or second heating.}
\end{figure}
Strategies in amorphous formulation design

Since crystallisation is the major problem associated with amorphous systems, inhibiting or delaying the crystallisation is the main goal in designing amorphous formulation. Very often, an excipient (usually a polymer) is added to stabilise the amorphous drug by forming an amorphous solid dispersion (ASD) \(^{32,57}\). The formulatability and functionality of the amorphous formulation depends on three interacting factors; (i) the compound itself, (ii) the excipient selected, and (iii) the method used for the preparation of amorphous formulation. Amorphous formulation design often involves the optimisation of these factors.

Current gaps in amorphous formulation research

In light of the background knowledge in the field and findings from our review of scientific papers on amorphous formulations published between January 2011 and December 2016, we identified the following knowledge gaps:

1. There was a lack of scientific reasoning in most of the amorphous formulation-related studies with regard to:
   - Selection of model compound. The scientific rationale for the selection was not clear (e.g., GFA classification, physicochemical properties, thermal behaviour).
   - Selection of excipient. Most of the studies quickly jumped into the development stage of the drug formulation. Excipients were almost always added without an explanation of their role in the amorphous formulation (e.g., as a stabiliser of the amorphous phase in solid-state, as an inhibitor of precipitation during supersaturation, as a dissolution enhancer, etc).
   - Selection of preparation method. Several preparation methods can be used to produce amorphous form of compounds, but the selection of method was rarely made with respect to the properties of the compound, e.g., physicochemical properties, thermal stability etc.) The amorphous solid material produced via different methods may also be different in terms of performance (e.g., supersaturation, stability).

2. There was a lack of performance assessment of the amorphous formulation, especially the long-term physical stability conducted in tandem
with supersaturation study. Studies on the implications of crystallisation during storage on supersaturation potential are rarely performed.

3. The majority of publications that reported *in vitro* dissolution/supersaturation assays used the large United State Pharmacopoeia dissolution apparatus, which requires large amounts of materials. Small-scale alternatives were rarely used.

4. Only a limited number of studies investigated large datasets. Large datasets, instead of case studies of one or only few compounds, are necessary to find statistical correlations or relationships between the studied variables. This in turn is useful when developing *in silico* model or any scientific tools used to predict formulatability.

5. Very few studies used newer, orthogonal techniques (such as molecular dynamic (MD) simulations) to explore, visualise, and understand the amorphous system from a molecular perspective.

**Motivation**

The direction of this thesis was steered by this background knowledge and the identified gaps in research methodology pertaining to amorphous formulation design. The main goal was to propose a strategic approach for rational design of amorphous formulations as a replacement for the conventional trial-and-error approach. This could be achieved by understanding what factors influence the crystallisation tendency of the amorphous drug, and *when and how* the amorphous drug crystallises. With this information, a proper selection can be made for compounds and excipients with appropriate physicochemical properties. This will produce amorphous formulations with favourable features and optimum performance.
Aims of the thesis

The overall aim of this thesis was to improve understanding of the crystallisation behaviour and crystallisation pathways or mechanisms of amorphous drugs to facilitate rational selection of drug and excipient(s) for amorphous formulations. The specific aims were to:

- Develop experimental and computational protocols to investigate the crystallisation mechanisms or pathways of amorphous drugs during dissolution (Paper I).

- Investigate factors affecting the glass-forming ability and long-term physical stability of spray-dried drugs stored under dry and humid conditions (Paper II).

- Delineate the use of differential scanning calorimetry in the glass-forming ability/glass stability classification (Paper III).

- Explore the effect of physical aging and crystallisation on supersaturation potential of amorphous drugs after long-term storage at humid condition (Paper IV).

- Investigate (i) the impact of drug loading on physical stability, supersaturation performance, drug/polymer miscibility and or mobility and (ii) the effect of physical aging and/or crystallization upon storage on supersaturation potential of spray-dried solid dispersions with hydroxypropyl methylecellulose acetate succinate (Paper V).
Materials and Methods

This section summarises the considerations undertaken with regard to materials and methods selection prior to the experimental work. Thereafter, the methodologies used in this thesis are briefly described. The readers are referred to the corresponding papers for more detailed description of the materials and methods.

Selection of model compounds

During the selection process of model compounds for this thesis, toxicity and hazard risk assessments were performed. The possible exposure to the researcher and the environment where the experimental work took place were considered. In general, compounds were selected with a low level of toxicity in their free form. All compounds were used as supplied by the manufacturer without further processing or modification.

Several specific criteria were considered for the selection of model compounds for Papers I to V. In Paper I, two pairs of analogous poorly water-soluble compounds, with different melting points, were selected. The difference (if any) in the crystallisation pathways of these analogues was studied. For Paper II, 30 glass-forming compounds were included initially, to study their glass-forming ability (GFA) upon spray-drying and long-term physical stability. The GFA was used as the main selection criterion. To ensure a dataset that was as random and as physicochemically diverse as possible, the selection criteria for Paper II did not take into account the compound solubility. In particular, calculated and measured physicochemical properties were considered: molecular weight (MW), octanol-water partition coefficient (logP), number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA), number of rotatable bond (RotB), polar surface area (PSA), glass transition temperature (T_g), crystallisation temperature (T_c), melting point (T_m), heat of fusion (ΔH_f) and entropy of fusion (ΔS_f).

However, four of the 30 compounds were excluded from Paper II because they exhibited promiscuous glass-forming behaviour, making it difficult to assign them to any glass-forming ability/glass stability (GFA/GS) classes. The four promiscuous glass-formers excluded from Paper II were then included in Paper III. The risk of coming across compounds with such behaviour was
briefly discussed when using differential scanning calorimetry as the screening method for GFA/GS classification. Seven compounds that were spray-dried as fully amorphous in Paper II were included in Paper IV, which investigated the supersaturation potential of those compounds upon long-term storage under humid condition. The experiments for Papers II and IV were performed concurrently. In Paper V, nine poorly water-soluble compounds were selected that could not be transformed to amorphous upon spray-drying (i.e. they spray-dried as fully crystalline) or were not stable during the long-term storage from Paper II. Sufficient solubility of compounds in acetone (≥ 1% w/w) was additionally considered for Paper V. The model compounds used in Papers I to V are summarized in Table 2.

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Selection of crystallisation inhibitors

Polymers of different categories are the most common crystallisation inhibitors used in stabilisation of amorphous compounds and/or formulations. Not only do they have a long-standing safety profile in different sorts of oral dosage forms, but they have also been used in several marketed amorphous-based products. Their effective role as crystallisation inhibitors in amorphous formulations has been demonstrated in several studies. In Paper I, polyvinylpyrrolidone K30 (PVP K30) and/or hydroxypropylmethylcellulose (HPMC) were used to stabilise supersaturation of all model compounds. In Paper V, spray-dried solid dispersions were prepared containing model compounds with hydroxypropyl methylcellulose acetate succinate (HPMC-AS). In addition to their reported positive performance as crystallisation inhibitors, the selected polymers were expected to be soluble in the solvent or solvent mixture used in these particular papers.

Preparation of samples

The samples used in Papers I, II, IV and V were prepared by spray-drying whereas in situ melt-quenching in a differential scanning calorimeter (DSC) was used to prepare the amorphous samples for Paper III. In Papers I to IV, the compounds were spray-dried and/or melt-quenched without any excipients or crystallization inhibitors. On the other hand, spray-dried solid dispersions were prepared containing HPMC-AS as the crystallisation inhibitor in Paper V. Two types of spray-dryer and parameter settings were used to prepare samples in Papers I, II, IV and V. Similarly, slightly different solvent systems were selected across these four papers. These are described in more detail in the following section.

Spray-drying

Solvent system for spray-drying solution

In general, organic solvents were used for the preparation of the spray-drying solution. In Paper I, the solution was prepared by dissolving each of the four model compounds in a standard solvent system. This solvent system consisted of a mixture of ethanol and acetone at 90:10% w/w. The amount of compounds dissolved was equivalent to 75% of their total solubility in the solvent mixture. In Papers II and IV, however, it was challenging to standardise the solvent or solvent mixture used to dissolve the model compounds. Some compounds were soluble in one solvent, while others dissolved better in another solvent. As such, either ethanol, acetone, or a mixture of ethanol and acetone at 90:10% w/w were chosen for Papers II and IV. In Paper V, acetone was the solvent of choice used across the entire dataset.
**Drug/polymer ratio of spray-dried solid dispersions**

In Papers I and V, spray-dried solid dispersions were prepared at different drug/polymer ratios. In Paper I, as low amount of PVP K30 as possible was used that could still produce completely amorphous solid dispersion (ASD). As such, an ASD of glipizide with PVP K30 was prepared at 50/50 % w/w ratio. In Paper V, spray-dried solid dispersions were investigated with 15/85, 25/75, and 50/50 % w/w of drug/polymer ratios. These ratios were selected on the basis of the calculated $T_g$ of the resulting spray-dried solid dispersion. The $T_g$ were calculated using Fox equation described below (Equation 2), from which fully amorphous solid dispersions were anticipated.

\[
\frac{1}{T_{gmix}} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}}
\]  

(2)

where $T_{gmix}$ is the glass transition of the drug/polymer mixture, $w$ is the weight fraction of component 1 and 2 respectively, and $T_g$ (1 and 2) is the glass transition temperature of each individual component.

**Spray-dryer**

A Büchi Mini Spray Dryer B-290 (Switzerland) was used to prepare samples used in Papers I, II and IV. The following spray-drying parameters used for these studies were: inlet temperature (55 °C), aspiration rate (75%), and pump rate (4 mL/min).

Paper V was performed in collaboration with Bend Research Inc./Lonza based in Bend, Oregon, USA. The solid dispersions were prepared using the Bend Lab Dryer at Bend Research Inc. facility. Prior to spray-drying, the predicted saturation at outlet or more commonly known as relative saturation (% RS) and $T_g$ of the amorphous solid dispersions were calculated. Based on these calculations, the following spray-drying parameters were selected: feed solution flow rate =30 g/min, atomisation pressure=10 psi, drying nitrogen flow rate=500 g/min and outlet temperature 35°C.

**In situ** melt-quenching in a differential scanning calorimeter

For Paper III, the samples were produced by subjecting the crystalline compounds to a standard heat-cool-heat cycle in a DSC. The first cycle involved heating the compounds at 10°C/min to slightly above their melting points, followed by a brief isothermal condition to allow complete melting of all the solid materials. Thereafter, the melts were cooled to -70°C at 20°C/min during the second cycle, after which they were immediately heated at 20°C/min during the third cycle. Depending on the thermal behaviour, compounds were classified according to their GFA/GS (discussed in detail in the Introduction).
Dynamic vapour sorption

In Paper I, a dynamic vapour sorption (DVS) was used to expose the spray-dried amorphous solid particles to high humidity relatively quickly. This mimicked the initial step in the dissolution process during which the surface of a solid particle is exposed to water. This technique allowed an investigation of solid-to-solid or particle-associated crystallisation, without fast dissolution of the solid. Prior to the exposure to high humidity, the samples were first dried at 10 to 20°C below their $T_g$ to remove any residual solvent. Then, the relative humidity (RH) was ramped from 0% to 98% within two minutes while the temperature was kept at 25°C. This condition was maintained for 24 hours. After the 24-hour exposure to 98% RH, the samples were analysed with a differential scanning calorimeter (DSC) and polarized light microscope (PLM). If the DSC and PLM analyses showed evident crystallisation, the sample was considered to have undergone solid-to-solid crystallisation.

Long-term physical stability study

The physical stability of spray-dried fully/partially amorphous compounds and solid dispersions was assessed in Papers II, IV and V. In Papers II and IV, a long-term (i.e. six months) physical stability study was performed on fully and partially amorphous compounds. These samples were stored under two different conditions that varied in their relative humidity (RH) – <5 % RH (dry) and 75% RH (humid), while the temperature was kept constant at 25°C. In Paper V, the storage stability of prepared spray-dried solid dispersions was investigated for four weeks at 25°C/75% RH and 40°C/75% RH. In these studies, samples were withdrawn at specified time points and solid-state changes were monitored by a combination of different solid-state characterisation techniques.

Solid-state Analyses

Several solid-state characterisation techniques were used to characterise the solid-state of the samples reported in the different papers included in this thesis. These are briefly described as follows.

Differential scanning calorimetry

In Papers I to V, the Q2000 DSC (TA Instruments, New Castle, DE, USA) was used to monitor solid-state changes in different samples (i.e., freshly spray-dried; after exposure to humidity in the DVS and stability chamber; and post dissolution). The following thermal properties were determined: $T_g$, $T_c$, $T_m$. 

25
T_m, ΔC_p, and ΔH_f. For these measurements, both a standard DSC and a modulated DSC were used. A standard DSC was most often the first choice due to its good sensitivity and relatively shorter measurement time (mainly used in Paper I). Nevertheless, modulated DSC was used when overlapping transitions occurred during heating, when small transitions were anticipated (e.g., the glass transition temperature), and/or when only a small sample size was available (Papers I to V). Additionally, a heat-cool-heat cycle in the DSC was used as a method to determine GFA/GS classes of compounds in Paper III.

**Polarised light microscopy**
A qualitative analysis of sample crystallinity, or lack thereof in Papers I and II was performed using a PLM (Olympus BX51 Tokyo, Japan). In short, samples were placed on a glass slide, dispersed in olive oil, and covered with a glass cover slip. The crystalline and amorphous samples were differentiated on the basis of their birefringence behaviour during the microscopic observation.

**Powder X-ray diffraction**
In Papers II, IV and V, the diffractograms of crystalline and spray-dried samples were analysed with a Bruker Twin-Twin powder X-ray diffractometer (Bruker, Coventry, United Kingdom). Samples that were fully crystalline, fully amorphous, or a mixture of crystalline and amorphous could be distinguished from their diffraction patterns. Additionally, emergence of any polymorph that differed from the reference crystalline samples could be identified. In short, a few milligrams of each sample were placed and compacted to give a smooth surface on a Si-plate. The diffraction pattern between a 2θ range of 5 and 40 was collected.

**Raman spectroscopy**
Crystallinity, amorphism and polymorphism can also be detected using Raman spectroscopy. An Rxn-2 Hybrid Raman Spectrometer (Kaiser Optical System Inc., Ann Arbor MI) – equipped with a laser (wavelength, λ = 785 nm, power = 400 mW) and a fiber-optic PhAT probe – was used to characterise crystalline (as supplied by manufacturer) and spray-dried samples in Papers I, II and IV. For the measurement, samples were placed on an aluminium sample holder and the spectra were collected in the wavenumber range between 100 and 1890 cm⁻¹. Further treatment of the Raman spectra was performed to allow semi-quantification on the sample crystallinity in Papers II and IV.
Semi-quantification of amorphous/crystalline content

In Papers II and IV, the changes in amorphous and/or crystalline content of stability samples were semi-quantified. Suitable Raman regions were selected, and the background were corrected and normalised. Using a classical least-squares equation, the proportion between crystalline and amorphous material in the samples was calculated using Equation 3:

\[
\vec{\text{synth}} = f_{\text{CR}} \cdot \vec{\text{CR}} + f_{\text{SD}} \cdot \vec{\text{SD}} = (1-f_{\text{SD}}) \cdot \vec{\text{CR}} + f_{\text{SD}} \cdot \vec{\text{SD}}
\]

where \(f_{\text{CR}}\) and \(f_{\text{SD}}\) are the weighted factors of the spectra of spray-dried and crystalline samples; \(\vec{\text{CR}}\) and \(\vec{\text{SD}}\) are the vector representations of the normalised crystalline sample (CR) spectrum and the normalised spray-dried sample (SD) spectrum, respectively; and \(\vec{\text{synth}}\) is the vector representation of the resulting synthesised spectrum. The factor \(f_{\text{SD}}\) was determined by a least-square curve fit of Equation 3 to the measured spectra. \(f_{\text{CR}}\) was determined as \(1 - f_{\text{SD}}\).

Scanning electron microscopy

The morphology of the spray-dried solid dispersions in Paper V were identified using a scanning electron microscope (SEM; Hitachi SU3500, Japan). Samples were applied on an adhesive surface of an aluminium stub, followed by sputtering with gold/palladium (AU/Pd) with a Hummer 6.2 sputtering system. Thereafter, images were captured at magnifications between 100x and 5000x. On SEM, spray-dried amorphous materials typically appear as collapsed spheres with smooth surfaces, whereas crystalline materials usually have sharp and well-defined edges and surfaces.

In vitro small-scale dissolution apparatus

A µDISS Profiler (Pion Inc, USA) was used to evaluate in vitro crystallisation behaviour of spray-dried samples and concentrated dimethyl sulfoxide (DMSO) drug stock solution under supersaturated condition in Papers I, IV and V. First, a standard calibration curve was constructed. This was followed by performing the dissolution studies in 3 mL phosphate buffer at pH 6.5. Temperature was maintained at 37°C. The instrument setup is illustrated in Figure 4.
Dissolution under non-sink conditions

The following investigations were performed in different studies included in this thesis: (i) the solution-mediated crystallisation of spray-dried amorphous compounds in Paper I and (ii) the impact of physical aging and/or crystallization on the supersaturation potential of spray-dried amorphous compounds and solid dispersions in Papers IV and V, respectively. During the dissolution, a non-sink condition was induced by adding the amount of compounds equivalent to their 10-folds apparent crystalline solubility. In Papers IV and V, the dissolution was followed for one and four hours, respectively.

Supersaturation via solvent-shift method

In Papers I and IV, a solvent-shift method was used to study the supersaturation and/or crystallisation behaviour of the supersaturated system. The supersaturation was generated via injection of a concentrated DMSO drug stock solution into the dissolution media. With this method, the dissolution step is avoided, allowing the researcher to identify solution-mediated crystallisation.

Supersaturation potential evaluation

The supersaturation potential assessment in Papers IV and V included the parameters shown in Figure 5 (i) apparent maximum concentration ($C_{\text{max, app}}$); and (ii) area under the curve (AUC) and crystallisation rate constant, (k). A GraphPad Prism version 8.1.0 for Windows (GraphPad Software, San Diego, California USA) was used for the calculation of these parameters.
Figure 5. Concentration–time profile of a supersaturated system showing the apparent maximum concentration ($C_{\text{max,app}}$), area under the curve (AUC), and crystallisation rate constant (k) and time to reach the apparent maximum concentration ($t_{C_{\text{max}}}$). Figure reprinted with permission from the publisher 103.

Molecular dynamics simulations

Molecular dynamics (MD) simulation was used as a complementary method in Papers I and V to probe the interaction between drug and polymer. In Paper I, the MD simulations were performed to understand the molecular interaction between drug and HPMC molecules in supersaturated solutions. In Paper V, molecular dynamic simulations were used to: (i) calculate Flory-Huggins interaction parameter of drugs and HPMC-AS at different drug loadings; (ii) estimate the molecular mobility in the absence and presence of water molecules; and (iii) explore the relationship between miscibility and/or stability with drug loading. The miscibility is reflected by Flory-Huggins interaction parameter value.

Univariate and multivariate analyses

In Paper II, potential influence of the compounds physicochemical properties on GFA and long-term stability was captured by performing univariate and multivariate analyses. The latter was performed with Simca, Version 15 (Umetrics, Sweden).
Statistical analyses

In Papers IV and V, an unpaired t-test and multiple t-test were performed on dissolution data of the fresh and aged/crystallised samples to determine the statistical significance of observed difference in $C_{\text{max, app}}$, AUC, and $k$, respectively. A p-value of <0.05 was considered statistically significant at a 95% confidence interval.
Results and Discussion

This section summarises the most significant findings of every paper included in this thesis. The readers are referred to the specific paper for more in-depth description and discussion of the findings. As each paper has a slightly different theme, the results of the individual papers are discussed separately (unless otherwise indicated) to ease readability.

Paper I: Crystallisation mechanisms of amorphous solid upon dissolution

Method to reveal crystallisation mechanism during dissolution

In Paper I, we proposed that the knowledge on crystallisation mechanism or pathway of amorphous solid during dissolution could be used in rationalising the stabilisation strategy for amorphous formulation. A study by Alonzo et al. reported that, upon dissolution, an amorphous compound crystallises either via solid-to-solid or solution-mediated crystallisation (as described in the Introduction)\(^{58}\). To permit investigation of these crystallisation pathways, we developed a systematic approach that combines solid-state characterisation and small-scale dissolution techniques (Figure 6). DVS, DSC and PLM were used to probe the solid-to-solid crystallisation whereas solution-mediated transformation was revealed via a dissolution study under non-sink condition. This protocol is relatively easy to use, requires small sample amounts, and has a short experimental time.
Indapamide, metolazone, glibenclamide and glipizide were selected as model compounds and HPMC and PVP (K30) as stabilising polymers. Each pair (indapamide-metolazone and glibenclamide-glipizide) is an analogue of the other, which means that, they have comparable molecular structures and selected because they differ mainly in their melting point (T_m). Initially, it was hypothesised that analogues with different T_m values differed in their crystal bonding strength. As such, the configurational enthalpy would vary upon amorphisation of the analogous pair. This enthalpy acts as the driving force for crystallisation of amorphous materials. Based on this relationship, the analogue with a higher T_m would most likely crystallise via the solid-to-solid mechanism, while the ones with lower T_m would crystallise through a solution-mediated one.

According to the systematic protocol established (Figure 6), the crystallisation pathway of the drug was determined based on when crystallinity was detected. The drug that undergoes solid-to-solid crystallisation would crystallise upon exposure to 98% RH in the DVS. In contrast, if the compound crystallises during dissolution, it suffers predominantly from solution-mediated crystallisation, which occurs upon the formation of supersaturation. The DSC thermograms and PLM images revealed that only glipizide crystallised via
solid-to-solid transformation while indapamide, metolazone and glibenclamide remained amorphous after exposure to 98% RH, indicating that their crystallisation was not induced via the solid pathway. Further, crystallisation pathways of the model compounds did not depend on their Tm. For instance, metolazone with a melting point of 268°C crystallised via solution-mediated instead of solid-to-solid crystallisation as hypothesised prior to the study.

Nevertheless, based on their concentration-time profiles, these three compounds seemed to suffer from solution-mediated crystallisation (Figure 7). The addition of 0.001% – 0.01% (w/v) HPMC into the dissolution medium successfully prevented the crystallisation of supersaturated solutions of indapamide and metolazone, whereas it only reduced the crystallisation rate for glibenclamide. The inhibition and/or deceleration of crystallisation resulting in stable supersaturation of these compounds strengthens the evidence that their crystallisation is predominantly induced by bulk supersaturation or solution-mediated.

Since spray-dried neat glipizide underwent crystallisation via solid-to-solid transformation, we attempted to stabilise it by producing amorphous solid dispersion (ASD) of glipizide with PVP K30, at a ratio of 50:50% (w/w). The presence of PVP K30 in the solid dispersion reduced, but did not completely eliminate, the solid-to-solid crystallisation of glipizide. However, the overall dissolution rate of the ASD was enhanced compared to the spray-dried neat glipizide, both in the absence and presence of HPMC.

Despite crystallising already after exposure to 98% RH, the spray-dried neat glipizide and its corresponding ASD exhibited comparable dissolution profiles to their respective freshly spray-dried samples, with or without the pre-dissolved HPMC in the dissolution media. The concentrations achieved were higher than the solubility of the unprocessed crystalline material used as the reference in this study. Furthermore, the dissolution profiles resembled a stable supersaturation, which certainly was not the case in the findings from DSC and PLM after exposure to 98% RH, which indicated that it underwent solid-to-solid crystallisation.

To further investigate the solid-state transformation occurring during dissolution, the post-dissolution sample of spray-dried glipizide was analysed and compared with unprocessed crystalline sample using Raman spectroscopy. The Raman spectra showed that glipizide transformed from the amorphous form to a polymorph different from the unprocessed crystalline one (Figure 8). This explains the higher solubility observed in the concentration-time profiles of spray-dried glipizide. This finding was also supported by the lower Tm detected by the DSC.
Figure 7. Dissolution profiles of (A) indapamide, (B) metolazone, (C) glibenclamide and (D) glipizide at 37°C. In every panel, • represents the respective crystalline drug in pure PbB₆.₅, — represents amorphous drugs in pure PbB₆.₅, ▲ represents amorphous drugs in PbB₆.₅ + 0.001% (w/v) HPMC and ■ represents amorphous drugs in PbB₆.₅ + 0.01% (w/v) HPMC. → represents ASD of glipizide: PVP K30 50:50% (w/w) in pure PbB₆.₅ and ◊ represents ASD of glipizide: PVP K30 50:50% (w/w) in PbB₆.₅+0.001% (w/v) HPMC. Each value represents the mean ± SD (n ≥ 3). Figure reprinted with permission from the publisher ⁷⁸.

Figure 8. Raman spectra of different glipizide samples: Unprocessed crystalline (green) and spray-dried after dissolution (red). Highlighted regions indicate Raman shifts of the peaks. Figure reprinted with permission from the publisher ⁷⁸.
Drug-polymer interaction: role of hydrogen bonding

To better understand the molecular interactions seen experimentally between drug and HPMC molecules in supersaturated aqueous system, MD simulations were performed. For this purpose, two representative model drugs – indapamide and glibenclamide – were selected on the basis of their experimental dissolution profiles in the presence of HPMC. Indapamide and glibenclamide exhibited stable and unstable supersaturation, respectively. The simulations showed that these two drugs possessed significantly different hydrogen bonding patterns (Figure 9). On average, indapamide formed more hydrogen bonding with HPMC than glibenclamide per-molecule. This suggests the important role of hydrogen bonding between drug and polymer in stabilising supersaturated solutions.

![Figure 9. Average number of hydrogen bonds (H-bond) per-molecule between individual indapamide molecules (IND–IND); indapamide molecules and HPMC (IND–HPMC); individual glibenclamide molecules (GLIB–GLIB); and glibenclamide molecules and HPMC (GLIB–HPMC), in three different systems with HPMC in the simulation box. Each bar in every dataset represents each system (from left to right) as follows: (i) unequal number of molecules; number of drug molecules corresponding to the 10-fold equilibrium solubility of indapamide (851 molecules) and glibenclamide (41 molecules), (ii) low number of molecules; number of molecules equal to the 10-fold equilibrium solubility of glibenclamide (41 molecules) for both indapamide and glibenclamide, and (iii) high number of molecules; number of molecules equal to the 10-fold equilibrium solubility of indapamide (851 molecules) for both indapamide and glibenclamide. The number of water molecules was fixed to 90% (w/w) in all systems. The error bars represent 95% confidence interval. Figure reprinted with permission from the publisher.](image-url)
Paper II: GFA and long-term physical stability

Selection of model compounds
In Paper II, we aimed to investigate (i) the influence of preparation method on the assessment of GFA and GS of compounds by comparing the GFA class of compounds prepared via spray-drying vs melt-quenching in the DSC, (ii) the physical stability of amorphous compounds prepared via spray-drying when stored at <5% RH (dry) and 75% RH (humid) conditions for six months (168 days) and (iii) the potential relationship between the long-term physical stability with glass forming ability (GFA) and/or physicochemical properties. Twenty-six previously reported glass-forming compounds (Class II and III) with diverse physicochemical properties were selected \(^1\). Priority was mainly given to poorly water-soluble glass-forming compounds, but compounds with satisfactory solubility from an administered dose perspective were also included.

Glass former vs. non-glass former
In the context of this study, a spray-dried compound was considered a glass former (GF) if the amorphous content of the sample was detectable with any or all of the solid-state analyses. This includes both completely amorphous and a mixture of the amorphous and crystalline. If the compound was spray-dried as a fully crystalline solid, it was classified as a non-glass former (nGF). Only 50% (n=13) of the compounds were GFs while the remaining 50% (n=13) were nGFs under the studied spray-drying condition. Of the 13 GFs, seven spray-dried as fully amorphous whereas six were amorphous-crystalline mixtures (Figure 10).

![Figure 10](image)

*Figure 10.* Pie charts showing (A) the glass-forming ability (GFA) of the model compounds produced via spray-drying method, and (B) the solid-state forms of the glass formers (GFs). The spray-dried compounds were classified as either non-glass formers (nGFs) or GFs. The GFs were further divided into fully amorphous or a mixture of amorphous and crystalline. Glass-forming ability/glass stability classification: melt-quenching vs. spray-drying.
Besides determining whether or not the spray-dried compounds were GFs or nGFs, we were also interested in comparing their GFA/GS classifications against the widely used systems of organic compounds established by Baird et al. via *in situ* melt-quenching in the DSC \(^ {86}\). The GFA/GS classes of the compounds obtained via melt-quenching and spray-drying are summarised in Figure 11. In general, the GFA/GS classes varied greatly for the two different preparation methods. Out of the total 26 model compounds, 16 compounds were classified as Class III while 10 compounds were classified as Class II upon melt-quenching.

When spray-drying was used instead, the GFA/GS classification of these compounds was more heterogeneous. Most compounds were down-classified in their GFA/GS classes, and none of the compounds were promoted to a higher GFA/GS classes when prepared by spray-drying. Also, a few Class III compounds via melt-quenching showed up as Class I when spray-dried. For the majority of the compounds, the GFA/GS classification is not only influenced by the preparation method used, but also by the specific conditions used for a particular method selected. Our findings are in good agreement with what has been reported by Van Eerdenbrugh *et al.* \(^ {87}\).

![Figure 11](image)

*Figure 11. The number of compounds in glass-forming ability/glass stability (a) Class III and (b) Class II according to in situ DSC melt-quenching compared to glass-forming ability/glass stability classes according to spray-drying, respectively. Pink, blue and green represent Classes I, II and III, respectively.*

**Long-term physical stability**

For the long-term stability assessment, only 13 of the compounds that were fully amorphous or formed a mixture of amorphous and crystalline were included. No further analyses were carried out on the remaining 13 compounds that were spray-dried as completely crystalline samples, except for the initial solid-state characterisation of their freshly spray-dried solids.

Figure 12 shows the three main stability patterns. The compounds were either: (i) stable under both dry and humid conditions, (ii) stable under dry conditions but unstable under humid conditions, or (iii) unstable under dry and humid conditions.

Among the studied compounds, indapamide and metolazone displayed exceptional stability when stored under both storage conditions. Glibenclamide,
hydrocortisone and hydrochlorothiazide, on the other hand, were stable when stored at dry condition but crystallised with different propensities at humid conditions. Affinity for water is predominantly influenced by the octanol-water partition coefficient (logP), which reflects the hydrophilicity and/or lipophilicity of a compound. These three compounds vary in their logP. Hydrochlorothiazide is the most hydrophilic (logP=-0.1) followed by hydrocortisone (logP=1.6) and glibenclamide (logP=4.8). This trend in hydrophilicity and/or lipophilicity agreed with the observed crystallisation tendency: hydrochlorothiazide > hydrocortisone > glibenclamide. The more hydrophilic compound has higher affinity for interaction with water than the lipophilic ones and hence, the amount of water absorbed may facilitate crystallisation.

Another interesting observation was that a rapid nucleation was not necessarily followed by rapid crystal growth, especially under dry storage conditions. This phenomenon was exemplified by sulfathiazole, prednisone, aripiprazole, glipizide and droperidol. These compounds crystallised completely at different time points when exposed to humid condition. However, the time to complete crystallisation, which reflects the crystal growth rate, was greatly suppressed under the dry condition, even though a detectable amount of crystals was already present in the sample upon spray-drying and/or after one-day storage. This was especially striking for aripiprazole and droperidol for which minimal crystallisation (≤ 15%) was observed throughout the 6-month storage at dry conditions (Table S1 of Paper II). These findings strengthen the assumption that interaction with water plays a vital role in influencing the physical stability of amorphous solids.

Probucol, which was spray-dried as a mixture of amorphous and crystalline, behaved rather differently from the rest of the compounds. The tendency to crystallise was similar regardless of the storage conditions. Probucol is very lipophilic (calculated logP=11.3) compared to the other compounds discussed above. Therefore, interaction with water is less likely to contribute to its crystallisation propensity. Nevertheless, the fact that it has a Tg (26°C) that is very close to the storage temperature (25°C), might have caused an increased molecular mobility and thereby enhanced the crystallisation tendency.

Influence of physicochemical properties on GFA and long-term physical stability

No strong correlation was shown between GFA and physicochemical properties of compounds. Nevertheless, glass formers tended to have relatively larger molecular weight (MW), a higher number of hydrogen bond acceptor (HBA), a higher polar surface area (PSA) a higher melting temperature (Tm), a higher crystallisation temperature (Tc), a higher glass transition temperature (Tg), and a higher reduced glass transition temperature (Trg) than the non-glass formers.
The increase in heat of fusion ($\Delta H_f$) and entropy of fusion ($\Delta S_f$) appeared to have a negative impact on GFA.

\[ \Delta H_f \text{ and } \Delta S_f \text{ impacted GFA.} \]

Figure 12. The 6-months (168 days) physical stability of the 13 spray-dried compounds included in the physical stability study. The stability of each compound is represented by color gradients over the study period: fully amorphous (green), different ratios of amorphous and crystalline mixtures (light to dark blue) and fully crystalline (red). The top and bottom bars for each compound represent the stability under dry conditions (<5% RH) and humid conditions (75% RH), respectively.

Nevertheless, no clear relationship between physicochemical properties and long-term physical stability could be established. A good GF does not necessarily make a stable GF, especially when humidity comes in play. In addition,
none of the individual physicochemical properties could predict physical sta-

bility.

These findings are in reasonable agreement with previous studies, where corre-

lations were found between long-term amorphous stability and physico-

chemical properties such as MW, HBD, T_g and ΔH_f. MW, HBD, and T_g

positively influenced physical stability whereas ΔH_f related to higher tendency
to crystallise and/or physical instability.

Paper III: The use of DSC in GFA/GS classification

Some compounds are difficult to classify into a specific GFA/GS class ac-

cording to the method suggested by Baird et al. This finding deserved more

attention as it may have important ramifications if in situ melt-quenching in
the DSC was the only method used to classify the GFA/GS classes of these
compounds. These might not affect only the GFA/GS classification of these
compounds, but also their overall performance (i.e., physical stability or su-

persaturation potential).

Model compounds and their purity

Acetohexamide, bifonazole, griseofulvin and piroxicam were included in Pa-

per III. However, griseofulvin, a widely used model compound in studies re-

lated to amorphisation and/or amorphous formulations, was the main focus of
the study. The thermal behaviour of four different batches of griseofulvin from
the same supplier, and a single batch of the other compounds were analysed
using the same DSC method, the same sample weight and by the same re-
searcher. Based on their thermal behaviour, the compounds were assigned a
GFA/GS class. Griseofulvin batches 1, 2 and 3 had a purity of 97-102% and
batch 4 had 95%. The purity of acetohexamide, bifonazole, and piroxicam
were 99%, 98% and 97%, respectively.

Griseofulvin: glass forming ability/glass stability classification

In this paper, the GFA/GS classifications were assigned according to the crys-
tallisation behaviour of the major transitions, ignoring the smaller ones. It is
imperative to evaluate the magnitude of the crystallisation peak to confirm
that it represents the major fraction of the sample under investigation.

The results classified griseofulvin as Class I and/or Class III, even though
the equipment and protocol were standardized. Griseofulvin batches 1 and 2
resulted in Class III when heated to 10°C above the melting temperature, in
all runs (Figure 13a and b). Batch 3 exhibited only Class I behaviour (Figure
13c), while batch 4 showed both Class I and Class III (Figure 13d). A tiny
crystallisation peak appeared during the cooling cycle in most of the griseofulvin samples showing a Class III behaviour. In some instances, this peak was associated with a small melting peak, approximately 10°C lower than the melting peak of the stable polymorph. This observation was not batch-dependent. Furthermore, for two out of three samples showing Class I behaviour, a small, but clearly visible, crystallisation peak was observed immediately before melting of the crystalline material.

Acetohexamide, bifonazole and piroxicam
Using the same procedure, the GFA/GS classes of acetohexamide, bifonazole and piroxicam were also examined. As with griseofulvin, categorising these compounds into one specific GFA/GS class was not possible. Based on their thermal behaviour upon heat-cool-heat in the DSC, these three compounds were classified as Class II and III. The GFA/GS classification of two compounds (acetohexamide and piroxicam) were dependent on the maximum temperature used for the first heating. Bifonazole, on a different end of the spectrum, became a Class II or III even though the same maximum temperature was used during the first heating. The GFA/GS classes for each of the compounds reported in this paper and in the previous studies are summarised in Table 3.
Figure 13. Thermograms for four batches – batch 1, 2, 3 and 4 of griseofulvin (denoted as A, B, C and D, respectively). Samples were subjected to a heat-cool-heat cycle in the DSC and melted to 10°C above the melting point during the first heating. Thermograms of the heat-cool-heat cycle are shown as follows; first heating (green), cooling (black) and second heating (red). Blue arrows indicate the small crystallisation and melting peaks. Two different runs of each batch are shown.
Table 3. Summary of GFA/GS classes of acetohexamide, bifonazole, griseofulvin and piroxicam in the current and previous studies. The classification was established on the basis of the thermal behaviour of the compounds when subjected to a heat-cool-heat cycle in a DSC.

<table>
<thead>
<tr>
<th>Compound</th>
<th>GFA/GS classes</th>
<th>Current study</th>
<th>Previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetohexamide</td>
<td>II / III</td>
<td>II</td>
<td>101</td>
</tr>
<tr>
<td>Bifonazole</td>
<td>II / III</td>
<td>II 86,101</td>
<td></td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>I / III</td>
<td>I, II</td>
<td>86,101,109,110</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>II / III</td>
<td>II 101</td>
<td></td>
</tr>
</tbody>
</table>

What causes promiscuous glass-forming behaviour?

The exact cause(s) of promiscuous GFA/GS behaviour in these compounds could not be identified. Particle surface has been suggested to influence the crystallisation behaviour in milled amorphous griseofulvin\textsuperscript{110}. As crystallisation is more likely to initiate on a particle surface, increased surface area often leads to a higher crystallisation tendency. The particle surface effects are nevertheless irrelevant in this case, since a single solid mass is produced during melt-quenching in the DSC. As long as complete melting is achieved during the first heating cycle, it can be assumed that no crystallites would remain that later could promote crystallisation during the cooling cycle. In this study, the same crystallisation behaviour was observed for griseofulvin when two different maximum temperatures above the $T_m$ were used during the first heat cycle, suggesting that melting was complete in both cases.

Upon melting of griseofulvin, no evidence of chemical reactions was seen from the thermogram. Nevertheless, some degree of degradation undetected by DSC cannot be excluded. The presence of degradants and impurities may have a significant effect on glass stability, even when they do not affect the $T_g$\textsuperscript{111}. Similarly, the presence of irregular or rough surfaces on the DSC pan cannot be entirely disregarded. Usually, nucleation and crystal growth can be induced on such surfaces\textsuperscript{72}.

In the case of griseofulvin, the presence and types of impurities in the crystalline samples seemed to be the only apparent factor that can account for the batch-dependent variation in chemical degradation (and hence glass stability). The impurity profile may differ among batches. A more in-depth study is needed to investigate the impact of different types and levels of impurities on stability and degradation behaviour upon heating.

Besides the impurities present in the crystalline powder as supplied by the manufacturer, impurities may also be introduced during sample preparation for the DSC runs. Finally, the stochastic nature of nucleation cannot be discounted as a contributing factor to the variations in the GFA/GS behaviour. A nucleation rate that coincides with the time scale of the DSC run may well explain the observed crystallisation tendency. It was also evident that such
promiscuous behaviour is not specific to grisoefulvin. In this paper, acetohexamide, bifonazole and piroxicam had the same behaviour.

Paper IV: Effect of physical aging and/or crystallisation on supersaturation potential

In this study, seven compounds that were fully transformed to amorphous after spray-drying in Paper II, were studied for their supersaturation potential. The performance of freshly spray-dried amorphous samples were compared with samples that had been stored at 75% RH until they were completely crystallised or for up to six months (168 days), whichever came first. The same batch of samples used in Paper II (stored at 75% RH) was used in Paper IV. The stability profiles are summarised in Table 4.

Table 4. Physical stability profile of spray-dried amorphous compounds upon storage at 75% RH and 25°C until they were completely crystallised or for up to 6 months (168 days) if no or incomplete crystallisation occurred.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stability after 6-months storage at 75% RH</th>
<th>Estimated crystalline content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indapamide</td>
<td>Good</td>
<td>0</td>
</tr>
<tr>
<td>Metolazone</td>
<td>Good</td>
<td>0</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>Moderate</td>
<td>6</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>Moderate</td>
<td>11</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Poor</td>
<td>100</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>Poor</td>
<td>100</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>Poor</td>
<td>100</td>
</tr>
</tbody>
</table>

Effect of long-term physical stability on supersaturation potential

The supersaturation profiles of the freshly spray-dried and aged and/or crystallised samples are shown in Figure 14. In general, all of the drugs displayed some degree of supersaturation, but no clear difference in supersaturation profiles could be observed between the fresh and aged and/or crystallised spray-dried drugs, except for ketoconazole. The completely crystallised spray-dried ketoconazole sample showed a similar dissolution profile as its crystalline form.

Indapamide, metolazone and glibenclamide displayed unstable supersaturation. A marked decrease in concentration was apparent after reaching an apparent maximum concentration ($C_{\text{max,app}}$) above the crystalline solubility. Conversely, the dissolution of hydrocortisone, hydrochlorothiazide, ketoconazole and sulfathiazole generated an apparently stable supersaturation for 60 minutes, at a concentration slightly higher than their crystalline solubility. It is noteworthy that, despite having a comparable estimated crystalline content of 6% and 11%, respectively, glibenclamide and hydrocortisone demonstrated
distinct supersaturation behaviours. It is evident, for these two compounds that the presence of small amount of crystals did not dramatically influence their supersaturation performance.

Figure 14. Concentration–time profiles of freshly spray dried (SD T₀) shown in blue circle. The crystallised and/or aged spray-dried compounds (SD Tₙ) are shown in red empty squares. The time point, n, depends on: (i) the time point at which the spray-dried samples completely crystallised, or (ii) the last time point of the stability study (i.e., 168 days) if crystallisation was incomplete or did not happen (as indicated in Table 4). The apparent crystalline solubility of each compound is shown as a black dashed line. Figure reprinted with permission from the publisher 103.

Cمامₚ and AUC ratio of fresh, aged and/or crystallised spray-dried solids

To further elucidate the differences in supersaturation profiles of fresh, aged and/or crystallised spray-dried samples, we performed statistical analyses on the Cمامₚ and AUC ratio (indicated as Rₐₚ and Rₐₚ, respectively) of both sample types for each compound (Figures 15). No significant difference in the Rₐₚ of the fresh and the aged and/or crystallised spray-dried drugs was observed, except for ketoconazole (Figure 15a). The completely crystallised spray-dried ketoconazole exhibited a 50% decrease in its Rₐₚ. In contrast, no significant impact was detected on the Rₐₚ of glibenclamide and hydrocortisone with estimated crystalline contents of 6% and 11%, respectively. Strikingly, hydrochlorothiazide, hydrocortisone and sulfathiazole,
which were fully amorphous freshly upon spray-drying, demonstrated very similar R_{C_{\text{max,app}}} to the partially or completely crystallised samples after 6 months storage at 75% RH.

![Graph](image)

*Figure 15.* (A) The C_{\text{max,app}} ratio (R_{C_{\text{max,app}}}) and (B) the AUC ratio (R_{\text{AUC}}) of the fresh and aged and/or crystallised spray-dried APIs at 10-folds supersaturation ratio. The freshly spray-dried samples are represented as SD T_0 (blue bars), whereas the crystallised and/or aged samples are denoted as SD T_n (red bars) since each of the compounds crystallised at different time points. At 95% confidence interval, a p-value of <0.05 is considered statistically significant. Figures modified and reprinted with permission from the publisher.

Except for ketoconazole, the extent of supersaturation (R_{\text{AUC}}) for the other compounds were not negatively influenced significantly by physical aging and/or crystallisation during storage (Figure 15b). Ketoconazole showed a 50% decrease in its R_{\text{AUC}}, which is in good agreement with the decline in R_{C_{\text{max,app}}}. A significantly positive impact, reflected by an increase in the R_{\text{AUC}}, was observed for metolazone. This may be attributed to the removal of electrostatic charges and irregularities via small adsorption of water on the particles surfaces. This minimizes particle agglomeration, leading to an increase in effective surface area for wetting and dissolution of solid particles. The partial or complete crystallisation of stored hydrochlorothiazide, hydrocortisone, and sulfathiazole did not seem to have a significantly negative impact on their R_{\text{AUC}}, compared with their freshly spray-dried fully amorphous counterparts. Of the seven drugs, glibenclamide achieved the highest supersaturation potential from amorphisation, whereas the other compounds demonstrated marginal or no improvement in their supersaturation potential.
Impact of crystallisation pathway on supersaturation potential

As seen in Figure 14, the fully amorphous and partially and/or completely crystallised stored samples of three drugs (hydrocortisone, hydrochlorothiazide and sulfathiazole) exhibited comparable concentration-time profiles. Such concentration-time profiles are usually associated with stable supersaturation, in which there is no ‘spring’ effect generated by supersaturation. There are two possible explanations for the observed supersaturation profiles of these drugs: (i) the crystallisation of amorphous solid during storage at 75% RH and crystallisation during dissolution followed the same pathway or mechanism, or (ii) the amorphous solid transformed upon dissolution into a metastable polymorph with higher solubility than the stable polymorph.

A solvent shift approach was used to probe the factors contributing to the supersaturation profiles observed with these model compounds, especially hydrochlorothiazide, hydrocortisone and sulfathiazole. In the solvent shift, the supersaturation is generated from a concentrated solution of the compound dissolved in dimethyl sulfoxide (DMSO) to evade the dissolution step. On the basis of the supersaturation profiles obtained via this method, one can get information on the predominating crystallisation mechanism or pathway. Our findings were coupled with solid-state analysis to probe any possible polymorphic changes during the dissolution and crystallisation process. The \( R_{\text{Cmax,app}} \) from the solvent shifts was examined and compared to the spray-dried ones (Figure 16).

Figure 16. The apparent C\(_{\text{max,app}}\) ratio (\( R_{\text{Cmax,app}} \)) of the solvent shift for fresh and aged and/or crystalline spray-dried compounds at ten-fold supersaturation ratios. The solvent shift is represented as SS (purple bars), the freshly spray-dried samples as SD \( T_0 \) (blue bars), and the crystallised and/or aged samples as SD \( T_n \) (red bars), since each of the compounds crystallised at different time points. At a 95% confidence interval, a p-value of <0.05 is considered statistically significant. Figure modified and reprinted with permission from the publisher.
In general, the $R_{C_{\text{max,app}}}$ of all drugs generated from concentrated stock solutions was higher than for those of the spray-dried samples. This indicates the significant role of solids during the dissolution in crippling the ability of the system to reach the highest possible $R_{C_{\text{max,app}}}$. Two different patterns in $R_{C_{\text{max,app}}}$ can also be seen in Figure 16. Glibenclamide, indapamide, ketoconazole, and metolazone reached the highest possible $R_{C_{\text{max,app}}}$ (i.e., 10-folds). However, the $R_{C_{\text{max,app}}}$ of hydrochlorothiazide, hydrocortisone and sulfathiazole was tremendously lowered (5-folds) compared to their crystalline solubility.

Crystallisation of glibenclamide, indapamide, ketoconazole and metolazone occurred from a relatively high degree of supersaturation. This suggests that they predominantly: (i) nucleated homogeneously from the bulk solution, and (ii) crystallised mainly via a solution-mediated pathway. Glibenclamide, indapamide and metolazone have been previously reported to undergo the same mechanistic pathway of crystallisation \(^{78}\).

Conversely, heterogeneous nucleation and solid-to-solid crystallisation seemed to be the predominating nucleation and crystallisation pathways for hydrochlorothiazide, hydrocortisone and sulfathiazole. They appeared to crystallise at a relatively lower supersaturation than the other four compounds. The large number of solid particles provides a large surface area for the solutes to nucleate on. Similarly, there is a greater likelihood for the formation of local supersaturation on the surface of particles as they are present in large numbers.

It is a known fact that nucleation proceeds more rapidly for compounds with higher solubility in the media \(^{72}\). This may also explain why the crystallisation of hydrochlorothiazide, hydrocortisone and sulfathiazole occurred at lower supersaturation compared with the other compounds. As solubility increases, the solute molecules have higher probability to see each other in the solution. It also causes more changes in the composition of the solution, hence, lowering the interfacial energy between the crystal and solution. As a result, the solute have more affinity for the crystal than the solution.

Role of polymorphism on supersaturation potential

Why did the supersaturation profiles of hydrochlorothiazide, hydrocortisone, and sulfathiazole appear like a stable supersaturation, even though they partially or completely crystallised upon storage? To investigate this, we performed solid-state analyses (i.e. Raman spectroscopy and DSC) on the solids collected after the dissolution experiments.

For all of the three compounds, the melting temperature and heat of fusion of the crystallised samples – either from storage at 75% RH or upon dissolution – were lower than the original crystal forms. This indicates the formation of metastable crystalline forms which, provides an explanation why the concentration of the crystallised samples of these compounds was higher than that
of the original crystalline. These analyses emphasised the importance of understanding the solid-state changes that may occur during dissolution. A stable supersaturation might not be the result of the amorphous nature of a given compound. Rather, it could be due to the crystallisation to a metastable polymorph with higher solubility.

**Crystallisation rate constant (k) and crystallisation kinetics**

The overall supersaturation decreases as nucleation and crystal growth ensue. As the system approaches equilibrium, the kinetics of nucleation and crystal growth decelerate. As a result, the thermodynamic aspects begin to dominate over the kinetic ones. The crystallisation rate constants (k) of hydrochlorothiazide, hydrocortisone and ketoconazole and sulfathiazole was not possible to calculate. This was due to the fact that their concentration-time profiles did not show the characteristic “spring” pattern associated with a supersaturating system. As such, no decline in concentration was detected after reaching the $C_{\text{max,app}}$. The crystallisation rate constant of the three remaining compounds are shown in Figure 17.

![Figure 17. Crystallisation rate constants, k (min\(^{-1}\)), of fresh and aged and/or crystallised spray-dried indapamide, metolazone and glibenclamide. Figure modified and reprinted with permission from the publisher.](image)

Out of these three compounds, only the crystallisation rate constant of the aged, spray-dried metolazone showed significant decrease compared with its fresh counterpart. No significant differences were measured for indapamide and glibenclamide. The crystallisation rate constant of the partially crystallised glibenclamide was expected to decrease more than the other two compounds since the system is approaching equilibrium as it crystallised. However, the small amount of crystalline material in the stored spray-dried glibenclamide samples did not affect the crystallisation rate. It was also unclear why the crystallisation rate constant of metolazone decreased significantly, despite it being stable upon storage.
Estimation of glass transition temperature and selection of drug loadings

Since drug loading is a major limitation in the development of amorphous solid dispersion (ASD) formulation, we wanted to investigate the impact of drug loading on the following: (i) drug/polymer miscibility, (ii) physical stability of the spray-dried solid dispersions under accelerated storage conditions, and (iii) supersaturation potential and stabilisation effect on supersaturation of HPMC-AS. Prior to spray-drying, the glass transition temperature ($T_g$) of the drug-polymer mixtures was estimated using the Fox equation (shown in the Materials and Methods section). Ideally, the drug-polymer mixtures produced should be molecularly dispersed and completely amorphous upon spray-drying. Therefore, it was important to ensure that the $T_g$ be reasonably higher than the outlet temperature. With these considerations in mind, drug-polymer mixtures were selected to contain 25% w/w (low) and 50% w/w (high) drug loading. However slightly different loadings were used for three of the total nine compounds (cinnarizine, clofocitol and fenofibrate), because they could not be produced as fully amorphous at drug loading >25% w/w. As such, 15% w/w (low) and 25% w/w (high) were used for these three compounds instead. The model compounds with their respective drug loadings are shown in Table 5.

Table 5. Model compounds with their respective drug loadings in the spray-dried solid dispersions.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug/HPMC-AS ratio (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15/85</td>
</tr>
<tr>
<td>Cinnarizine</td>
<td>✓</td>
</tr>
<tr>
<td>Clofocitol</td>
<td>✓</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>✓</td>
</tr>
<tr>
<td>Bifonazole</td>
<td>✓</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>✓</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>✓</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>✓</td>
</tr>
<tr>
<td>Probucol</td>
<td>✓</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>✓</td>
</tr>
</tbody>
</table>
Solid-state forms of spray-dried solids dispersions

All of the drugs – except bifonazole, probucol and sulfamerazine at 50% w/w drug loading – were spray-dried as fully amorphous solid dispersions in the studied drug loadings. These findings are quite striking given that the $T_g$ of all the solid dispersions was estimated to be higher than the outlet temperature. From the $T_g$ vs. outlet temperature standpoint, crystallisation of the drug during spray-drying was unlikely, since the estimated $T_g$ of the spray-dried solid dispersions were at least 28°C higher than the outlet temperature. This could be due to the poor predictability of Fox equation and/or poor miscibility between the drug and polymer, which could lead to phase-separation and physical instability of the spray-dried solid dispersions.

Physical stability under accelerated storage conditions

As shown in Table 6, cinnarizine, clofoctol, clotrimazole, griseofulvin and ketoconazole remained completely amorphous after four weeks of storage at 25°C/75% RH and 40°C/75% RH, independent of the drug loadings. However, clofoctol, clotrimazole and ketoconazole underwent some morphological changes even though they stayed amorphous throughout the stability study period. Visual observation and SEM images showed that the solid particles aggregated to form one large hardened solid (Figure 18). This phenomenon was observed for the spray-dried solid dispersions containing higher drug loading stored at 40°C/75% RH.

The remaining compounds crystallised to different extent when exposed to the two storage conditions. Of the nine model compounds, sulfamerazine displayed very poor stability at both drug loadings and storage conditions, especially at high drug loading (50% w/w) and the more extreme storage condition (40°C/75% RH). As expected, the crystallisation during storage took place mainly in solid dispersions that already contained some degree of crystallinity upon spray-drying. In such solid dispersions, the crystallisation process was induced by the presence of the seed crystals, and accelerated by the exposure to high temperature and high humidity.
Table 6. Stability of different drug-polymer mixtures at different loadings (15%, 25% and 50% w/w) after four weeks of storage at 25°C/75% RH and 40°C/75% RH.

<table>
<thead>
<tr>
<th>Drug</th>
<th>15%</th>
<th>25%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25°C</td>
<td>40°C</td>
<td>25°C</td>
</tr>
<tr>
<td>Cinnarizine</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Clofocitol</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Bifonazole</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Probulc</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
<td>![Pie Chart]</td>
</tr>
</tbody>
</table>

*Solid particles fused to form large and hard aggregated particles. The circles represent the proportion of amorphous (green) and/or crystalline (red) in the drug-polymer mixtures. Green only = fully amorphous, red = crystalline and green + red= different proportion of amorphous and crystalline depending on the size on the pie represented by each color.

Estimation of Flory-Huggins interaction parameter

A series of molecular dynamics (MD) simulations was performed to calculate the interaction parameter (χ) using Flory-Huggins theory. According to this theory, the interaction parameter value reflects the miscibility of the produced drug-polymer mixture. This can be used to explain if the different stability profiles are influenced by a possible phase separation in the solid dispersion systems. The lower the interaction parameter, the higher the miscibility is between the two components.

Usually, a mixture is considered to be phase separated if the \( \chi \geq 0.5 \) \(^{116}\). From our calculations, the interaction parameter of all our drug-polymer mixtures under dry condition fell below 0.5. Therefore, phase separation was not expected in any of the drug-polymer mixtures used. This indicates that, the varying stability patterns observed was unlikely to be due to phase separation of the solid dispersions.
Figure 18. Scanning electron microscopy images of the spray-dried solid dispersions of clofoctol, clotrimazole and ketoconazole with high drug loading. Left: the freshly spray-dried sample. Right: the four-week stability sample of the respective spray-dried samples stored at 40°C/75% RH. High loading is 25% w/w for clofoctol and 50% w/w for clotrimazole and ketoconazole.

Interestingly, an inverse linear correlation ($R^2=0.84$) between logP and in silico calculated Flory-Huggins interaction parameter ($\chi$) was observed from the dataset (Figure 19). It can be seen from the figure that, the higher the logP, the lower the Flory-Huggins interaction parameter ($\chi$). This indicates that, the more lipophilic the compound is, the better the miscibility with HPMC-AS. Such correlation has been previously predicted, but to the best of our knowledge, it has not been validated or shown experimentally and/or computationally.
Determination of molecular mobility and miscibility as a function of drug loading

Stability (or instability) is often associated with an increase in molecular mobility in solid dispersion systems\textsuperscript{118-120}. As such, we attempted to find correlation between the stability of spray-dried solid dispersions observed experimentally and their molecular mobility after annealing observed from MD simulations. In the simulations, the mobility of the drug molecules was determined by calculating the root mean square fluctuation (RMSF). This was performed for systems across a range of drug loadings between 5% w/w and 75% w/w for three compounds: griseofulvin – represents the stable system at both experimentally observed drug loadings (25% and 50%); probucol – represents a system that is stable at 25% but unstable at 50% and sulfamerazine – represents a system that is unstable at both loadings (see Table 6). No significant difference in mobility was revealed for these three compounds with changes in drug loading. However, there was an increasing trend in mobility with the increase in drug loading.

Further, a computational model was applied to probe the possible influence of drug loading on miscibility of the solid dispersion system. The same model compounds (griseofulvin, probucol and sulfamerazine) were used to represent systems with different physical stability (explained in the above section). It was revealed from the MD simulations that the Flory-Huggins interaction parameter value significantly increased with an increase in drug loading beyond 5% and 15% w/w.
MD simulations of drug-polymer mixture in the presence of water molecules

In order to investigate the mobility of drug and polymer in the presence of humidity, water molecules were added to the simulation box to make 5% weight of the entire system. The water was added either randomly with drug and polymer molecules or as a layer on the top of the randomly mixed drug and polymer. Mobility was measured by determining the radial distribution function (RDF), number of contacts between atoms of different groups of molecules (API, polymer, water) and root-mean square fluctuations (RMSF). Again, griseofulvin, probucol and sulfamerazine were used as model compounds to represent amorphous systems with different stability profiles.

In the systems with probucol, the drug molecules preferred to interact with themselves than with polymer and water, forming what appeared like a phase-separated system. Sulfamerazine tended to create a network spreading throughout the whole box. The drug molecules in the network were not only connected among themselves, but were also in contact with water and polymer molecules. Griseofulvin formed small clusters of aggregates without observable complete phase-separation.

The number of contacts between drug molecules with themselves (API-API), with polymer molecules (API-polymer) and water molecules (API-water) for the three model compounds used in this MD simulations are shown in Figure 20. It can be seen from this figure that, probucol is highly hydrophobic, while sulfamerazine have high affinity for water molecules. Griseofulvin is somewhat intermediate in its affinity for polymer and water molecules. It is postulated that the mesh created by sulfamerazine molecules might represents crystallisation of the drugs in the presence of the HPMC-AS and water. Griseofulvin aggregates do not form a single cluster, but rather remain separated with polymer-water mixture. That could be a mechanism to preserve the amorphous form. While for probucol, a complete phase separation occurred at both drug loadings, which indicates of possible crystallisation.
Figure 20. The ratio of the active pharmaceutical ingredients (APIs) contact with themselves, polymer and water out of a total 100%. A contact is represented by the appearance of an atom of one molecule at a distance of less than 0.6 nm from an atom of another molecule.

**Supersaturation performance**

The second part of this study assessed the supersaturation stability of spray-dried solid dispersions. These included: (i) the supersaturation profiles as a function of drug loading, and (ii) the impact of physical aging and/or crystallisation on supersaturation potential.

**Supersaturation vs. drug loading**

The supersaturation behaviour of the spray-dried solid dispersions are shown in Figure 21 for cinnarizine, clofoctol and fenofibrate (15% and 25% w/w drug loading), and Figure 22 for the remaining six compounds (25% and 50% w/w drug loading). The supersaturation behaviour was different across the model compounds. In general, the increase in drug loading did not always result in a better or worse supersaturation performance. In most cases, no significant difference was observed in the apparent maximum concentration (C_{max,app}) and extent of supersaturation (area under the curve, AUC), except for the C_{max,app} of probucol and the AUC of griseofulvin and probucol. Nevertheless, it is noteworthy that some compounds displayed distinguishable supersaturation profiles.

For instance, clofoctol (calculated logP=8.1) and probucol (calculated logP=11.3) which are highly lipophilic did not have the typical spring-parachute \(^{116}\) associated with the supersaturation pattern of an ASD. Instead, the concentration increased very slowly over four hours until it reached a concentration higher than the crystalline solubility. It is known that a quick dissolution of the drug often leads to a sudden increase in concentration (generating
supersaturation), which is the driving force for precipitation (and crystallisation) from a supersaturated solution. However, for clofoctol and probucol, the dissolution proceeded very slowly. Therefore, no sudden increase in concentration occurred that could act as the driving force for crystallisation. This shows that the rate of dissolution – as opposed to the rate of crystallisation – is an important kinetic factor that determines which of the two processes dominates. These observations are notable for clofoctol and probucol with 15% w/w and 25% w/w drug loading, respectively. The high loading (50% w/w) did not seem to have a solubility advantage for probucol, for which the concentration achieved was comparable to the crystalline solubility.

![Figure 21](image)

**Figure 21.** Supersaturation profiles of freshly spray-dried cinnarizine, clofoctol and fenofibrate spray-dried solid dispersions at 15% w/w (dark brown circles) and 25% w/w (light brown empty circles) drug loading. Black dashed-lines indicate the apparent crystalline solubility and the red dotted-lines show the maximum supersaturation if 100% of the drug is dissolved at 10-folds supersaturation ratio.

Different supersaturation profiles of bifonazole solid dispersions were observed at 25% w/w and 50% w/w drug loadings. For the first two hours, the solid dispersion with lower loading exhibited a superior supersaturation than the one with high loading. Thereafter, the concentration of both bifonazole loadings became similar to each other. No difference was observed in the supersaturation profiles of clotrimazole at either loading, which appeared to be stable supersaturation. A concentration about five-folds higher than the crystalline solubility was reached in both loadings.

Cinnarizine, fenofibrate, griseofulvin and ketoconazole exhibited comparable supersaturation patterns. All of them reached a $C_{\text{max,app}}$ close to, or equivalent to, 100% release of the drugs, but the $C_{\text{max,app}}$ decreased rather quickly, indicating an unstable supersaturation and/or solution-mediated crystallisation.

No comparison of sulfamerazine supersaturation profiles could be made. The spray-dried dispersion with 50% w/w drug loading produced a cloudy solution, making it difficult for the instrument to measure the concentration correctly. The cloudiness was most likely due to rapid crystallisation upon dissolution and possibly formation of micro- or nano-structures or aggregates.
Figure 22. Supersaturation profiles of fresh bifonazole, clotrimazole, griseofulvin, ketoconazole, probucol spray-dried solid dispersions at 25% w/w (light brown empty circles) and 50% w/w (red circles) drug loading. Black dashed-lines indicate the apparent crystalline solubility and the red dotted-lines show the maximum supersaturation if 100% of the drug is dissolved at 10-folds supersaturation ratio.

Supersaturation vs. physical aging and/or crystallisation

After four weeks of storage at 25°C/75% RH and 40°C/75% RH, no significant difference was detected in the supersaturation behaviour of cinnarizine, clofocort and fenofibrate, regardless of the drug loading (Figure 23). The minor crystallisation detected for 25% w/w fenofibrate after four weeks storage at 40°C/75% RH did not significantly affect the supersaturation profile and crystallisation kinetics during dissolution. Similarly, morphological changes of clofocort did not worsen the supersaturation potential to any significant extent, even though the initial rate of dissolution was slightly lower than for the fresh sample, most likely due to the increase in particle size of the stored clofocort solid dispersion.

The impact of physical aging and/or crystallisation on the supersaturation behaviour of six compounds with drug loading of 25% w/w and 50% w/w is shown in Figure 24. In general, the supersaturation of each compound was affected differently by aging and/or crystallisation during storage. The most striking difference was observed in the supersaturation profile of the spray-dried solid dispersion with 50% w/w ketoconazole stored at 40°C/75% RH. The concentration climbed to the C\text{max,app} slower than the other samples, followed by a slower decrease in concentration. This was most likely due to the increase in particle size because of the hardening and formation of large aggregated solid particles. This in turn leads to a slow dissolution of the large
aggregates compared to the smaller and more segregated freshly spray-dried solid particles. This finding also indicates that the rate of crystallisation is partly dependent on the rate of dissolution.

It is also interesting to observe that the significantly higher $C_{\text{max,app}}$ and AUC of probucol solid dispersion with 25% w/w drug loading compared to the fresh one. The exact reason is unclear. However, it has been reported that electrostatic charges of particles are eliminated by a small amount of water adsorption $^{112,113}$. This reduces the tendency of particles to form agglomerate, especially during contact with water upon dissolution, which might explain the higher supersaturation attained by stored probucol solid dispersion with 25% w/w drug loading compared to the fresh one.

As for sulfamerazine, only the solid dispersions with 25% w/w drug loading could be measured and compared. As discussed in the previous section, the instrument could not measure the concentration correctly due to cloudiness in the solution. The $C_{\text{max,app}}$ and AUC of 25% w/w sulfamerazine was tremendously jeopardized by the crystallisation upon storage, and was significantly lower than the one from the fresh sample (i.e. about 2.5 times lower). Samples stored at both conditions reached a $C_{\text{max,app}}$ comparable to the crystalline solubility.

![Figure 23](image-url)

*Figure 23.* Supersaturation profiles of freshly spray-dried solid dispersions and spray-dried solid dispersions after 4-week storage at 25°C/75% RH and 40°C/75% RH of cinnarizine, clofocntl and fenofibrate at 15% and 25% w/w drug loading. Black dashed-lines indicate the apparent crystalline solubility and the red dotted-lines show the maximum supersaturation if 100% of the drug is dissolved at 10-folds supersaturation ratio.
Figure 24. Supersaturation profiles of fresh spray-dried solid dispersions and spray-dried solid dispersions after 4-weeks storage at 25°C/75% RH and 40°C/75% RH of bifonazole, clotrimazole, griseofulvin, ketoconazole, probucol and sulfamerazine at 25% and 50% w/w drug loading. Black dashed-lines indicate the apparent crystalline solubility and the red dotted-lines show the maximum supersaturation if 100% of the drug is dissolved at 10-folds supersaturation ratio.
Conclusions

This thesis demonstrates that a combination of experimental and computational tools can be used to improve understanding of the crystallisation behaviour and crystallisation pathways or mechanisms of amorphous formulations. This knowledge can be used to guide a rational preparation of viable and well-functioning amorphous formulations. Specifically, the following conclusions are highlighted:

- An easy-to-use and systematic protocol combining solid-state analytical methods and a small-scale dissolution apparatus can be used to reveal whether amorphous compounds crystallise via solid-to-solid or solution-mediated mechanism during dissolution (Paper I).

- Hydrogen bond patterns between drug and polymer play a significant role in stabilising supersaturation generated by dissolution of amorphous drug (Paper I).

- The glass forming ability/glass stability (GFA/GS) classification of drug compounds are influenced by preparation methods. Spray-drying produces more heterogeneous distribution across the GFA/GS classes than the in situ melt-quenching using differential scanning calorimetry (DSC) (Paper II).

- When stored at dry or humid condition, amorphous compounds display different long-term physical stability, even though they belong to the same GFA/GS class (Paper II).

- The crystallisation of amorphous compounds during storage is induced and accelerated by interaction with humidity/water (Paper II).

- There is a risk in using DSC as the only screening method for GFA/GS classification of compounds. Promiscuous glass formers are difficult to assign to a specific GFA/GS class, despite standardisation of experimental protocol and a single operator running the experiment (Paper III).
- Physical aging and/or minimal crystallisation (≤ 11%) after long-term storage of amorphous compounds have a marginal impact on supersaturation potential and crystallisation kinetics during dissolution, as demonstrated by most of the model compounds (Paper IV).

- The supersaturation potential of amorphous compounds is greatly influenced by the mechanisms or pathways of crystallisation during dissolution (Paper IV).

- The effect of (i) drug loading on physical stability and supersaturation performance and (ii) physical aging and/or crystallisation upon storage on supersaturation potential of spray-dried solid dispersions containing HPMC-AS are drug-specific. The stabilisation mechanism of this polymer ranges from inhibition of solid-to-solid crystallisation to inhibition of solution-mediated crystallisation (Paper V).

- The calculated Flory-Huggins interaction parameter indicates good miscibility of all model compounds with HPMC-AS in the studied drug loadings (Paper V).

- For some drugs, via MD simulations, an increased mobility and formation of different molecular arrangement and complexes were observed in the presence of water molecules, suggesting possible phase separation and/or crystallisation of the drugs in the solid dispersion systems (Paper V).
Contributions of the Thesis

This thesis work has contributed to an improved understanding of amorphous system emphasising on the crystallisation behaviour and the crystallisation pathways or mechanisms involved. The major strengths of this thesis work are:

1. The development of a systematic protocol by using several complementary and small-scale methods to reveal and discriminate different crystallisation pathways via which amorphous solid crystallises during dissolution. The protocol is easy-to-use and demands small sample amount, which is of great advantage in the early stage of drug development, where availability of compounds is limited.

2. The studies were performed using a relatively large number of model compounds with diverse physicochemical properties. While it is of high interest to carry out a comprehensive investigation on a single model compound, a large dataset provides information on trends and overall understanding of important aspects in the development of amorphous formulations. These aspects include compound properties, crystallisation behaviour, and performance. Such general findings are impossible to extract in studies that use a single model compound.

3. The efforts to investigate long-term physical stability and the implications of physical aging and the extent of solid crystallisation during storage on the supersaturation performance of amorphous compounds. Very limited number of studies have, till date, investigated both solid crystallisation during storage and supersaturation potential in the same study with more than one model compound.

4. The use of molecular dynamics (MD) simulations to probe molecular understanding of amorphous system in its solid-state form and under supersaturated condition. In this thesis, the interaction between drug and polymer and mobility at the molecular level were successfully investigated with this computational approach. The MD simulations were also used to estimate the miscibility of drug and polymer components by calculating the Flory-Huggins interaction parameter. These simulations are robust because they allow compounds in a large dataset to be studied simultaneously. MD simulations provide useful complementary information around amorphous formulation that cannot be probed or challenging to investigate experimentally.
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