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The development of a Multiple linear regression model for aiding formulation development of solid dispersions

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Abstract

As poor solubility continues to be a problem for new chemical entities (NCEs) in medicines development the use and interest in solid dispersions as a formulation-based solution has grown. Solid dispersions, where a drug is typically dispersed in a molecular state within an amorphous water-soluble polymer, present a good strategy to significantly enhance the effective drug solubility and hence bioavailability of drugs. The main drawback of this formulation strategy is the inherent instability of the amorphous form. With the right choice of polymer and manufacturing method, sufficient stability can be accomplished. However, finding the right combination of carrier and manufacturing method can be challenging, being labour, time and material costly. Therefore, a knowledge based support tool based upon a statistically significant data set to help with the formulation process would be of great value in the pharmaceutical industry.

Here, 60 solid dispersion formulations were produced using ten, poorly soluble, chemically diverse APIs, three commonly used polymers and two manufacturing methods (spray drying and hot-melt extrusion). A long term stability study, up to one year, was performed on all formulations at accelerated conditions. Samples were regularly checked for the onset of crystallisation during the period, using mainly, polarised light microscopy. The stability data showed a large variance in stability between, methods, polymers and APIs. No obvious trends could be observed.

Using statistical modelling, the experimental data in combination with calculated and predicted physicochemical properties of the APIs, several multiple linear regression (MLR) models were built. These had a good adjusted R^2 and most showed good predictability in leave-one-out cross validations. Additionally, a validation on half of the models (eg. those based on spray-drying models) using an external dataset showed excellent predictability, with the correct ranking of formulations and accurate prediction of stability.

In conclusion, this work has provided important insight into the complex correlations between the physical stability of amorphous solid dispersions and factors such as manufacturing method, carrier and properties of the API. Due to the expansive number of formulations studied here, which is far greater than previously published in the literature in a single study, more general conclusions can be drawn about these correlations than has previously been possible. This thesis has shown the potential of using well-founded statistical models in the formulation development of solid dispersion and given more insight into the complexity of these systems and how stability of these is dependent on multiple factors.

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Abbreviations

<i>ANN</i>	Artificial neural networks
<i>API</i>	Active pharmaceutical ingredient
<i>ASA</i>	Water accessible area
<i>AUC</i>	Area under curve
<i>BCS</i>	Biopharmaceutics classification system
<i>ClogP</i>	Calculated lipophilicity
<i>DSC</i>	Differential scanning calorimetry
<i>FBDD</i>	Fragment based drug discovery
<i>FH</i>	Flory Huggins
<i>GFA</i>	Glass forming ability
<i>HBA</i>	H-bond acceptors
<i>HBD</i>	H-bond donors
<i>HLB</i>	Hydrophilicity – lipophilicity balance
<i>HME</i>	Hot melt extrusion
<i>HPMCAS</i>	Hydroxyl propyl methyl cellulose Acetate Succinate
<i>HTS</i>	High through-put screening
<i>Int</i>	Intercept
<i>IQR</i>	Inter quartile range
<i>K</i>	constant
<i>LOD</i>	Limit of detection
<i>logS₀</i>	Intrinsic water solubility
<i>LOOCV</i>	Leave-one-out cross validation
<i>MD</i>	Molecular dynamics
<i>MLR</i>	Multiple linear regression
<i>molPol</i>	Molecular polarizability
<i>mp</i>	Melting point
<i>MSE</i>	Mean square error
<i>MW</i>	Molecular weight
<i>nAliph</i>	Number of aliphatic atoms
<i>nAliphRings</i>	Number of aliphatic rings
<i>nAr</i>	Number of aromatic atoms
<i>nArRings</i>	Number of aromatic rings
<i>nAtom</i>	Number of atoms
<i>nBond</i>	Number of bonds

<i>nC</i>	Number of carbon atoms
<i>NCE</i>	New chemical entity
<i>nChir</i>	Number of Chiral atoms
<i>nCl</i>	Number of chlorine atoms
<i>nCov</i>	Number of covariates
<i>nF</i>	Number of fluorine atoms
<i>nHet</i>	Number of heteroatoms
<i>nHet/C</i>	Number of heteroatoms divided by number of carbons
<i>NMR</i>	Nuclear magnetic resonance
<i>nN</i>	Number of nitrogen atoms
<i>nO</i>	Number of oxygen atoms
<i>nRing</i>	Number of rings
<i>nRot</i>	Number of non-terminal rotatable bonds
<i>nS</i>	Number of sulphur atoms
<i>PCA</i>	Principal component analysis
<i>PEG</i>	Poly ethylene glycol
<i>PLM</i>	Polarised light microscopy
<i>PLS</i>	Partial least squares
<i>PVP-VA</i>	Poly vinyl pyrrolidone - vinyl acetate
<i>QSAR</i>	Quantitative structure–activity relationship
R^2_{adj}	Adjusted R^2
<i>RF</i>	Random forest
<i>RH</i>	Relative humidity
<i>SEDDS</i>	Self-emulsifying drug delivery system
<i>SOL</i>	Polyvinyl caprolactam polyvinyl acetate polyethylene glycol
<i>SVM</i>	Support vector machine
<i>T_g</i>	Glass transition temperature
$T_{g\text{ meas}}$	Measured T_g
$T_{g\text{ pred}}$	Predicted T_g using Fox equation
<i>tPSA</i>	Topological polar surface area
<i>vdW-SA</i>	Van der Waals surface area
<i>wt</i>	weight
<i>XRPD</i>	X-ray powder diffraction

Chapter 1 - Introduction

1.1. Oral drug delivery

Oral drug delivery, specifically solid oral dosage forms e.g. tablets and capsules, is the preferred administration route to date. Such formulations are generally stable, comparatively easy to produce and have low cost of goods [1,2]. Additionally, solid oral dosage forms are the preferred dosage forms by patients due to easy administration. Historically, oral delivery has been the most important route of administration and generally show good patient compliance. Although, the clear dominance has subsided in recent years, due to the rise of biopharmaceuticals, it is likely to continue to play a crucial role in the future [3].

1.2. Poor drug solubility

Poor water solubility of many drug compounds in development remains a problem. Compared to around 40% of marketed active pharmaceutical ingredients (APIs), up to 90% of new chemical entities (NCEs) are classified as poorly soluble [4]. This trend towards more lipophilic drugs has been attributed to drug discovery programmes based on target biology, organisational factors such as high throughput screening and historical libraries, and the chemical processes used for synthesis [5–8]. A notable difference in lipophilicity has been observed between NCEs that failed phase I clinical trials and those that progressed to phase II. Higher lipophilicity was associated with higher attrition rates [9]. Guidelines such as Lipinski's 'rule of five' give indication where poor solubility could be expected due to physicochemical properties of the API. The rule of five states that poor solubility and or permeation, thus poor absorption, can be expected if a compound breaks any of four rules (see Table 1) [10]. Despite general guidelines, the current inclination in the industry is towards larger and more lipophilic APIs, both properties which negatively affect the water solubility [6]. Around 30% of lead compounds in development do not meet the criteria for the rule of five [5].

A review of patents from 18 pharmaceutical companies in 2000-2010 showed that only very small decreases in logP and no improvements in MW when compared to marketed products. This indicates that better HTS libraries and general guidelines on drugability do not achieve marked advancements on drug physicochemical properties [7]. However, the drug discovery and development process is generally very long, around a decade. Thus, improvements due to changes in discovery and development processes can take some years to have a noticeable difference.

Table 1 Lipinski's rule of five. APIs that break any of these rules are expected to have poor bioavailability.

*Physicochemical
property*

Molecular weight (MW)	< 500 Da
Lipophilicity (logP)	< 5
H-bond donors	< 5
H-bond acceptors	< 10

Fragment based drug discovery (FBDD) is a relatively new method of finding and optimising lead compounds in drug discovery. It has become increasingly popular in recent years with the first drug discovered using FBDD, vemurafenib, being launched in 2011, with further examples in clinical trials [11,12]. FBDD typically starts by screening a relatively small library of fragments, where each fragment generally has a low molecular weight of ≤ 300 g/mol. The fragments undergo screening for binding with target proteins, using biochemical assays such as nuclear magnetic resonance (NMR), surface plasmon resonance (SPR), mass spectrometry (MS) and X-ray screening [13–15]. The binding mode of the fragments that show affinity is often studied through cocrystallisation. The fragments are then further optimised using the information from the screenings and binding modes. The fragments are enlarged through linking, growing or merging to take advantage of further possible interactions with the protein binding site. As the lead optimisation starts with a small drug fragment, generally with low MW and logP, it provides

good opportunity to control these. Several comparisons of FBDD and HTS have shown that the latter derives significantly larger and more lipophilic leads than FBDD [7,13]. Importantly fragments are generally more soluble than larger molecules, as good solubility is required for the biophysical screening assays. Furthermore, by screening fragments rather than whole drug-like libraries, larger chemical space can be covered with substantially fewer compounds, which leads to more chemical diversity in lead compounds [14,16]. This could indicate that in the future, fewer NCEs will be poorly soluble or even that during optimisation molecular trades connected with certain formulation types, such as glass forming ability, could be built in. This remains to be seen.

1.2.1. Challenges connected with poor solubility

NCEs with poor aqueous solubility carry increased risk of failure during pre-clinical and clinical phases. A substantial percentage of poorly soluble, highly potent NCEs do not reach the clinical stage due to failure to adequately improve solubility [17,18]. Poor solubility can cause inconsistent and poor exposure in both animal models and clinical trials. This is partly due to the simple formulations often used in early *in vivo* and clinical trials. First in human formulations tend to be very simple, such as drug in capsule or drug in bottle, both of which are just the API with no or minimal excipients. Recently it has been suggested that formulation development should be done earlier in the process to increase the chance of success of developing poorly soluble compounds [19,20]. A review of the attrition rates in the pharmaceutical industry paints a daunting picture. An industry average success rate of APIs that reach the market is only 4.1 %, this is even worse in some therapeutic areas such as Alzheimer's disease where it is only 0.5 % [21]. Failure in phase II clinical trials is mainly due to lack of efficacy (51%) but also strategy (29 %) and safety (19 %) [22]. When compared to phase III, almost 90% of failed trials were due to efficacy (66 %) and safety (21 %) [23]. In some cases, lack of efficacy could be due to poor or inconsistent exposure of the NCE caused by poor solubility.

1.3. Methods to improve solubility

Several methods can be exploited in order to increase solubility and dissolution of poorly soluble APIs, as has been reviewed by Williams *et al.* and Kawabata *et al.*, [24,25]. An overview of these and when they can be used is depicted in Figure 8 and Figure 1. Firstly, chemical alterations of the drug molecule, such as introducing polar groups or making a more soluble prodrug. Secondly, modifications to the crystal form can increase solubility, these include polymorphs, salt formation, cocrystals, solvates or hydrates, and amorphous form. Additionally, particle size reduction, nanoparticles or nanocrystals, lipid formulations, cyclodextrin complexations and solid dispersions are commonly used.

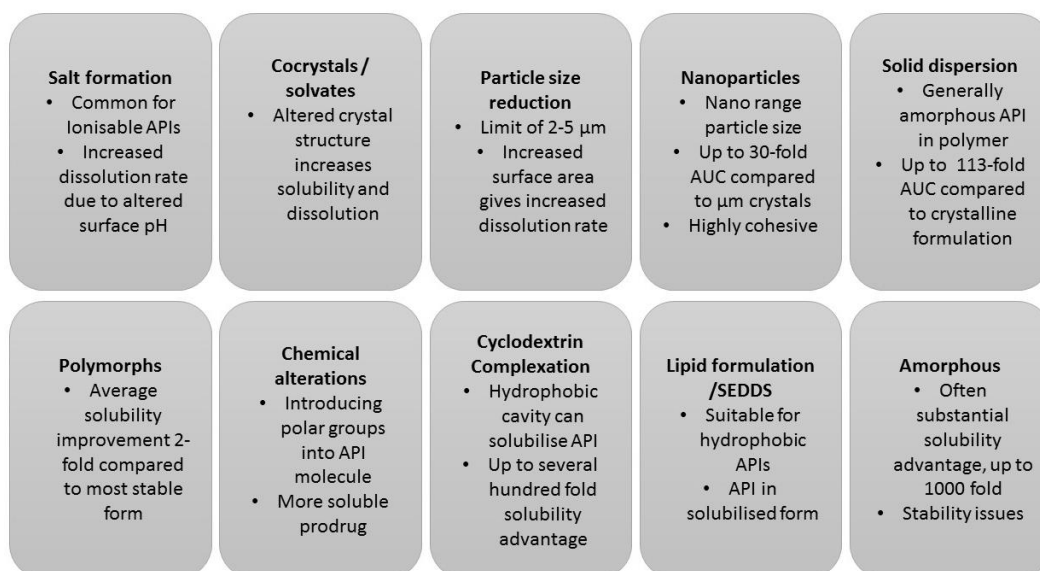


Figure 1 Overview of routes for improving API solubility

A crystal is made from molecules packing together in a certain orientation to form a stable three-dimensional crystal lattice. Molecular interactions within the crystal lattice, including van der Waals, π - π stacking, electrostatic interaction, H – bonding and ionic interactions, determine the stability of the crystal. The stronger the overall molecular interactions within the crystal lattice, the more energetically stable it is. Therefore, the most stable crystal has the least molecular mobility and the lowest solubility [24]. Thus a crystal form that does not have the maximum molecular interaction possible, will be a less stable polymorph. Polymorphs are meta stable

crystal forms of the API which have different physicochemical properties than the most stable form. Due to the lower energy of the crystal in metastable polymorphs the solubility is higher [25]. A review of polymorph solubility showed an average polymorph solubility ratio of 1.7, where most polymorphs had less than twofold solubility advantage to the most stable form [26].

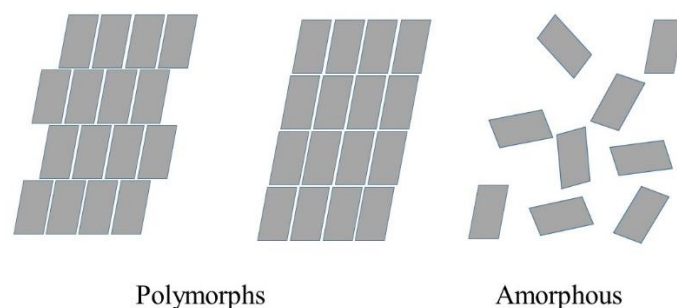


Figure 2 schematic figure showing polymorphs and amorphous form arrangements

For ionisable APIs, salt formation is one of the simplest and thus cost effective ways to improve solubility for a poorly soluble API. It is therefore a commonly used method for ionisable compounds. Salts are formed when stable ionic bonds are formed between ionised API and a counter ion with a difference in pK_a of three [25,27,28]. The salt has a higher dissolution rate due to the altered pH the counter ion causes in the diffusion layer of the particle. There are a number of counter ions that can be used, some are generally allowed in all quantities while others can only be used in more sparing quantities due to their possible toxic effects [29].

Cocrystals are similar to salts, crystals containing at least two different molecules. However, contrary to salts, both molecules are in the neutral state, interacting non-ionically, see schematic overview in Figure 3 [28,30,31]. They can therefore be used for neutral APIs as well as ionisable ones. An example of a cocrystal former that can be used is flavonoids [31]. The same definition can be applied to hydrates and solvates where the second type of molecule, in addition to the API, is either water (hydrate) or a solvent molecule. All the above, salts, cocrystals and solvates, use a second, added molecule, to alter the physicochemical properties of the solid state of the API, in order to improve the solubility [25,32].

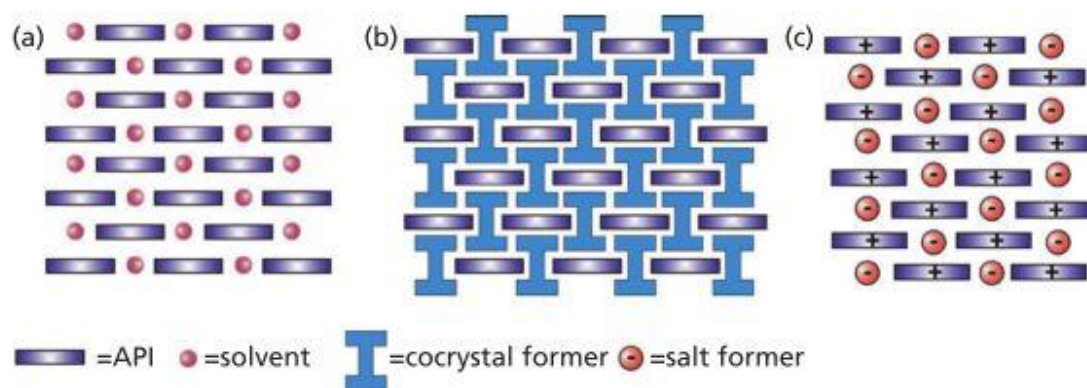


Figure 3 A schematic figure of a) hydrate / solvate, b) cocrystals and c) salts, from [32]

Compared to the crystalline form, the amorphous form has no strong intermolecular interactions and no long-range three dimensional order of molecules (schematic overview in Figure 2). Thus the solubility and dissolution is greater than for the crystalline counterpart. The solubility advantage of an amorphous over crystalline has been shown to be in some cases very substantial, up to several hundred fold is possible. However, similar to polymorphs, they are inherently unstable and will convert, over time, to a more stable crystalline form [6,33,34].

Particle size reduction increases dissolution rate through increased surface area of the particle. Through conventional milling the particle size generally cannot be reduced further than to 2-5µm. However, the small particle size needed for improved dissolution can cause problems such as agglomeration, poor flow and adhesion. Although more complex, size reduction down to nanometre range can be achieved through nanomilling. Nanoparticles, both amorphous and crystalline, are very cohesive and need stabilising excipients such as surfactants to prevent aggregation. Nanocrystals have shown up to 30 fold area under the curve (AUC) compared to a crystalline formulation with micrometre particle size [2,24,25,35].

Complexation with cyclodextrins has also been successfully used to improve solubility. Cyclodextrins are macrocyclic oligosaccharides with hydrophilic exterior and an inner hydrophobic cavity. The cavity provides lipophilic micro

environment which API molecules of suitable size can penetrate. No covalent bonds are formed and once in solution the molecule can exit the complex. The solubility increase from this complexation can be substantial, a literature review showed up to several hundred fold increase [4,24,25,36].

Lipid formulations such as self-emulsifying drug delivery systems (SEDDS) are very useful solubilising technique for very hydrophobic APIs. Lipid formulations generally contain the API in solubilised form in addition to oil, surfactants, co-surfactant and co-solvent. SEDDS form fine emulsions when mixed in an aqueous solution. Depending on the size of the oil droplets in the water they are classed into self – microemulsifying drug delivery systems (SMEDDS) and self – nanoemulsifying drug delivery systems (SNEDDS) [24,25,37].

Solid dispersions are generally made with amorphous API and polymer carrier, thus sometimes called amorphous solid dispersions. The API is nano- or even molecularly dispersed in amorphous form in the polymer carrier. The polymer stabilises the amorphous form both in the solid state and in dissolution to retard crystallisation. Solid dispersions have shown up to 113-fold increase in AUC compared to a crystalline formulation or physical mixture [2,24,25,38].

1.4. Solid dispersions

Solid dispersions have historically been produced with a range of carriers, both crystalline and amorphous, such as urea and sugars (crystalline) or polymers. They have furthermore been made both with crystalline and amorphous API. Now they are generally produced using amorphous API and polymer. The polymer can be highly water soluble, for immediate release, alternatively, water insoluble or swellable polymers, for extended release. Solid dispersions have been the topic of many studies and reviews such as by Leuner and Dressman, and Baghel *et al.* [39,40].

The increase in effective solubility and dissolution rate of APIs in solid dispersions compared to the crystalline form are due to many factors. Firstly, there is significant solubility advantage of the bulk solid connected with amorphisation, as mentioned above. Additionally, as the API is nano- or molecularly dispersed the particle size is extensively decreased compared to crystalline formulation. Furthermore, when using a hydrophilic polymer carrier these can improve the wettability and dissolution rate of the API. Amphiphilic polymers can form micelles when dissolved, which further solubilises the API and prevents crystallisation [2,38,41,42].

Amorphous solid dispersions show a single glass transition temperature (T_g) and no melting peaks, when characterised by differential scanning calorimetry (DSC) and an absence of peaks, amorphous halos, as associated with amorphous material in X-ray powder diffraction (XRPD).

The biggest hurdle to overcome in solid dispersion formulation is the inherent instability of the amorphous form. As the API crystallises the enhancement in solubility and bioavailability is lost. However, suitable stability can be obtained with a solid dispersion with optimal choice of polymer carrier, process method and storage conditions. The miscibility of the amorphous API in the polymer is critical for a stable formulation. Stability and achievable drug load in the solid dispersion will depend on how easily the API is dissolved and stabilised by the polymer. Heat sensitivity and solubility of API in common volatile solvents can impact choice of manufacturing method.

1.4.1. Glass forming ability

A classification system was developed based on how easily APIs can be changed to their amorphous form, or their glass forming ability (GFA). APIs are classed into three categories, class I - non-glass formers, class II – non-stable glass formers and class III – stable glass formers. The classification is based on when an APIs crystallises in a heat-cool-heat cycle in differential scanning calorimetry (DSC).

Class I crystallises during first cooling, class II during the second heat ramp while class III remain amorphous [43]. GFA is considered a tool for formulation development, indicating whether an API is suitable for an amorphous formulation. GFA classification has been observed to be independent of manufacturing method [44]. A recent review of marketed solid dispersions and amorphous formulations showed that a majority of the APIs were class III, stable glass formers, with a few class II and only one non-stable glass former [45]. Some effort has been put into finding correlations between physicochemical properties and the GFA and predicting GFA [6,46–48].

1.4.2. Manufacturing methods

Several techniques have been used to prepare solid dispersions as reviewed by Vo *et. al.*, and Vasconcelos *et. al.* [2,38,49]. These are generally divided into solvent evaporation and melting methods. Solvent methods employ in most cases organic, volatile solvents which solubilises both polymer and API. Once the solvent is rapidly removed what remains is a solid dispersion. Examples of solvent evaporation methods are freeze drying, vacuum drying, rotary evaporation and spray-drying [50–55]. The melting methods melt together polymer and API into a homogenous mass which is then cooled to produce the solid dispersion. These include hot melt extrusion and melt quenching [56–58]. Other techniques such as, mechanical methods, using neither solvent nor heat, have also been used, for example ball milling and cryo-milling or a combination of melt and solvent methods, see Figure 4 [44,59]. The most commonly used methods in the pharmaceutical industry to produce solid dispersions are spray-drying and hot melt extrusion (HME).

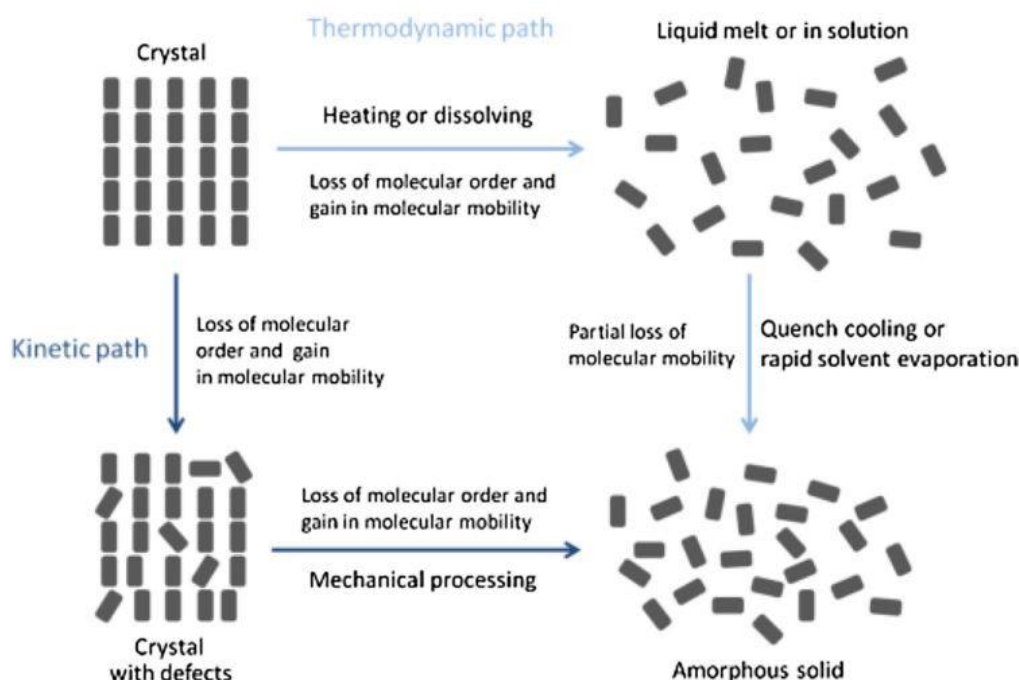


Figure 4 overview of the different types of methods used to produce solid dispersions, melt and solvent methods (thermodynamic) and mechanical (kinetic) methods from [44]

1.4.2.1. Hot melt extrusion

Hot melt extrusion (HME) is the most commonly used melting method in solid dispersion production and has thus been well studied and reviewed [60]. The starting material for hot melt extrusion is a physical mixture of the API and polymer carrier. The powder blend is fed into the HME through a hopper. The HME consists of a long, heated barrel that contains two screws which either co-rotate or counter-rotate. The barrel is heated, generally above the T_g of the polymer to soften or melt the polymer so the API is dissolved in the polymer. The screws knead together the molten mass to create a homogenous mixture. Depending on the size of the HME the mixture might be circulated for a certain amount of time to ensure proper mixing, see Figure 5. The mixture rapidly cools on extrusion to form a glass. This can be further processed such as pelletized or milled, for use in *e.g.* tableting or as a filling in capsules [41,60,61].

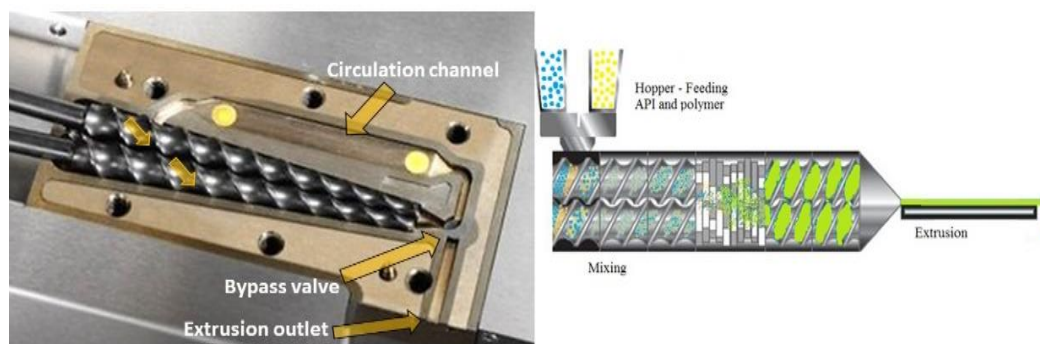


Figure 5 left shows the inside of a HAAKE MiniLab HME. The whole bronze area is heated and the two screws co-rotate to knead the molten polymer and API into a homogenous mass. The mixture is circulated until fully mixed. Once the bypass valve is turned the mixture is extruded and becomes a solid glass when cooled. Right, shows a schematic picture of a HME with either co- or counter- rotating screws, adapted from [62]. In larger HME there is no circulation as the longer barrel achieves adequate blending.

Main advantages of HME are that it is a continuous process which facilitates scale-up and it does not require any use of solvent, and thus there is no issue with residual solvents. Furthermore, it has a small footprint in the laboratory and minimal environmental impact [40].

Disadvantages of HME are that it is not applicable to heat labile API and generally not used for APIs with high melting temperatures ($> 200\text{ }^{\circ}\text{C}$) [6]. Furthermore, the minimum batch size is large, even in table top models, at least a few grams.

1.4.2.2. Spray-drying

Spray-drying is the most commonly used solvent evaporation method in the pharmaceutical industry. It has been widely studied and reviewed [42,63]. The spray-drying process requires the API and polymer to be in a common solution of a volatile solvent, such as ethanol, methanol, dichloromethane or acetonitrile. The solution is pumped into the drying chamber through the nozzle, where it is atomised and sprayed through warm gas or air. The heat, above the boiling point of the solvent, causes rapid evaporation of the solvent from the solution, leaving small homogenous particles of solid dispersion. The particles can be spherical or shrunk, raisin like, depending on the drying conditions [64]. The particles dry in the drying chamber and are collected at the bottom of the cyclone, see Figure 6.

Due to short exposure to heat in the drying chamber, small amounts of solvent remains in the collected product, typically around 2-10% wt. Thus secondary drying is generally needed to remove the residual solvent [42,65].

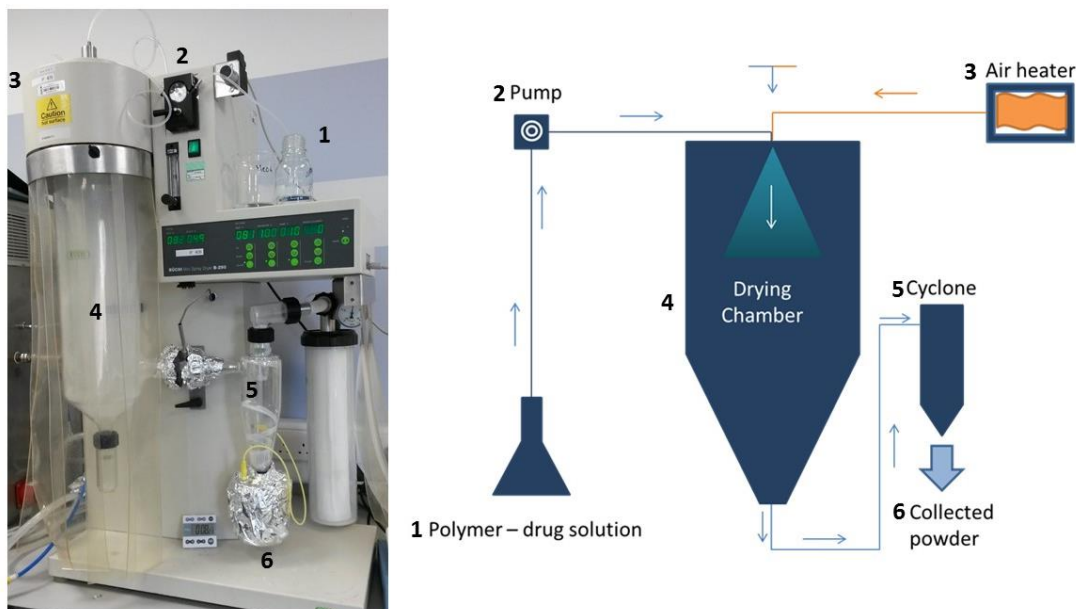


Figure 6 left, a Buchi spray drier, a typical lab-scale system. Right, a schematic figure of a spray-dryer.

The main advantage of spray-drying (and other solvent evaporation methods) is the absence of high temperatures, and therefore this method is suitable for heat labile APIs. Spray drying is generally performed below the melting point of API but the inlet temperatures depends on the solvent used. Spray-drying is a material sparing method, where batch size of down to approximately 0.5g can be achieved. However, it can be scaled up to a batch size of around a ton. The small particle size and generally porous material produced by spray-drying further improve dissolution rate compared to the larger, solid particles of HME [40,65].

Disadvantages of spray-drying (and other solvent evaporation methods) are mainly a result of the solvents. Finding a suitable solvent which can solubilise both API and polymer can be challenging as often the API is hydrophobic whilst the polymer

is hydrophilic. Organic solvents are generally toxic which can be problematic during production as well as leading to extremely little tolerance for residual solvents in final products. Furthermore, explosion risk and the environmental impact of the solvents must be considered. Complete removal of all residual solvents is nearly impossible but through secondary drying most of the solvent is evaporated. Residual solvents, in addition to being toxic, can decrease stability of the solid dispersion, acting as a plasticiser. [38,41,65].

1.5. Support tools in formulation development

As poor solubility is often known early in the development of an NCE it is important to be able quickly to identify which formulation strategies are available and optimal in each specific case. Instead of dealing with solubility issues on a project-by-project basis, relying to some degree on trial and error, a predictive support tool would be very valuable for giving indications of possible solutions early without having to conduct many experiments. Quantities of NCEs are usually limited early in development and such a tool could greatly optimise the use of what is available.

The optimal formulation for each specific drug depends on numerous factors, including the required dose, administration route, shelf life, manufacturability, and the physicochemical properties of the drug. Choosing the optimal formulation for a drug is therefore not a straightforward process and a poor choice of formulation can have devastating effects on the development process for a drug, such as leading to poor clinical data, which in turn can necessitate drug re-formulation and prolonged clinical trials– or even termination of the project [66]. Currently there are several ways that can be used to select the best formulation route for a new drug candidate. These differ in complexity and efficiency and range from trial and error formulations, small scale *in vitro* or *in vivo* experiments, guidance maps, and decision trees to complex computational modelling tools. An overview of support tools used in formulation development is shown in Figure 7. Support tools in formulation development have been the focus of a few reviews such as by Kuentz *et al.* [3], DeBoyace and Wildfong, and Fridgeirsdottir *et al.* [17,67].

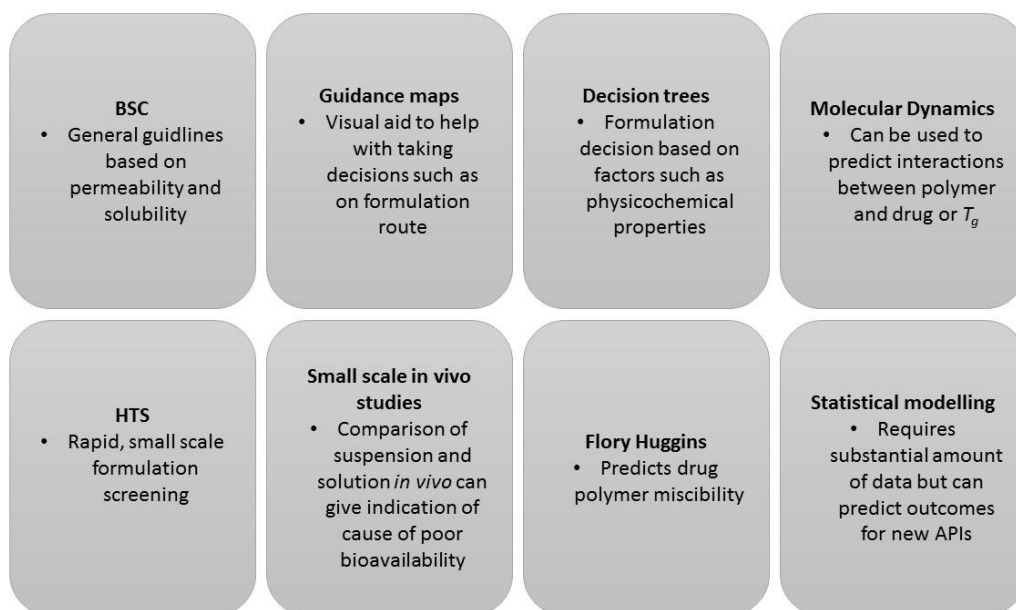


Figure 7 An overview of support tools that can be used in the formulation development process.

1.5.1. Classification systems

The Biopharmaceutics Classification System (BCS) is a basic tool used in drug development. The BCS classes drugs into four categories based on solubility and permeability. APIs which are 90% absorbed or more in humans are classed as highly permeable. The criteria for high solubility are that the highest dose strength of the API is soluble in 250 mL of aqueous solution over the pH range 1–7.5 [68–70]. The classification system can be used in formulation development, to indicate available routes to increase bioavailability (Figure 8) and attempts have been made to increase the value of this tool by adding further parameters to it [25,71–73]. An example of this is the developability classification system by Dressman and Butlers which changed the solubility criteria to include a biorelevant media and an increased volume. Another system, still under construction is the manufacturing classification system which is designed to aid with choosing a formulation route for an API. The system is, similar to the BCS, based on 4 classes with increasing risk associated with each class. The classes are (I) direct compression, (II) dry granulation, (III) wet granulation, and (IV) other technologies, which includes melt granulation and liquid dispensing technology [74].

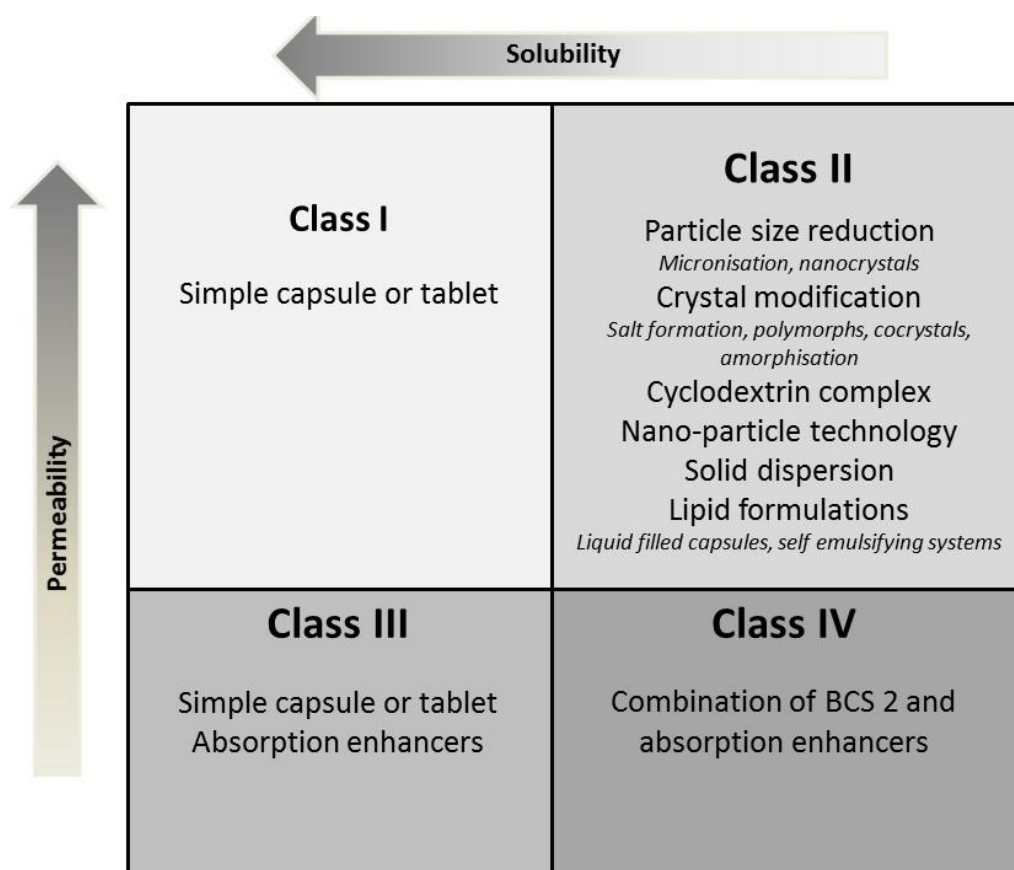


Figure 8 The BSC classification of an API can be used to indicate a suitable formulation route, adapted from [25].

1.5.2. High through-put screening

In any formulation development process there is a set of most commonly used excipients which are first tested with a NCE. If those are unsuitable there is a long list of additional excipients, ratios, and processing conditions that can be tested. If no suitable formulation with adequate solubility is found, the drug candidate cannot advance in the development cycle. In such cases a high through-put screening (HTS) approach can be valuable, where it is possible to test a large number of combinations of excipients or formulations using only a very small quantity of API. Furthermore, it is less labour- and time-intensive than performing the same experiments on a larger scale. Several HTS methods have been employed to produce formulations for poorly soluble compounds, using solvent evaporation methods which can then be characterised for redispersibility, solubility, particle size and more [75–79].

1.5.3. Guidance maps

Guidance maps are developed to give indications about the probability of an event. They are a visual aid to show the probability of a method being successful. For instance, a guidance map could address the probability of a solubilisation technique to give sufficient absorption for a drug to be efficient, give indication of which technique is most suitable for a drug, or even how much drug loading can be used for a drug. An example of guidance maps is depicted in Figure 9. These can be used to aid with the decision of formulation route depending on dose and physicochemical properties of the API [80]. Another guidance map, based on experimental results of 130 APIs, indicates drug loading capability of spray-dried solid dispersions in HPMCAS [81].

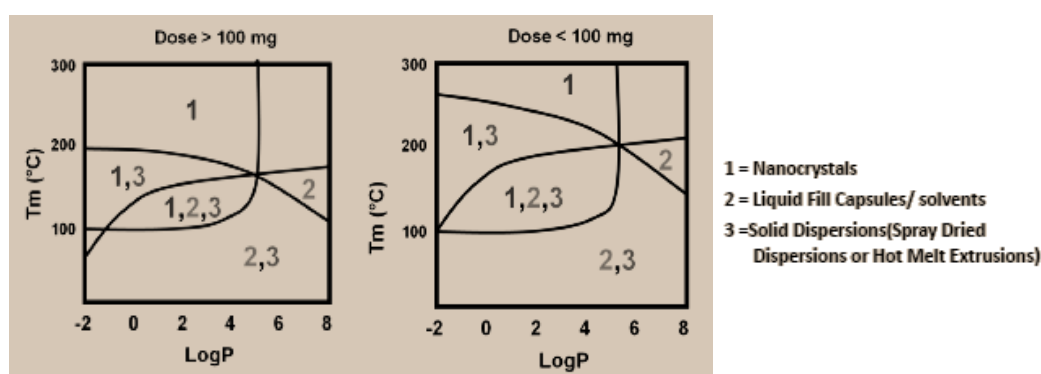


Figure 9 An example of a guidance map which can be used to guide choice of formulation route for API depending on melting point, logP and dose. 1) nanocrystals, 2) liquid fill capsules / solvents and 3) solid dispersions

1.5.4. Decision trees

A decision tree is a support tool where each decision in the formulation selection is represented as a step in a flow chart. Thus the whole decision tree (or flow chart) will guide one through the steps that need to be considered when choosing the optimal formulation for an API. Each tree is built with a specific task in mind, such as the development of an oral formulation for poorly bioavailable drugs [18], an *in vitro* formulation [82], choosing processing method for solid dispersion formulation [49,83], a first-in-man formulation [66,84], or even depending on type and timescale of formulation process [20]. These trees vary in complexity: some are quite simple with few steps and outcomes, while others have many steps and

possible outcomes, Figure 10. Most are based on the properties of the API and are quite general, and not developed for a specific API.

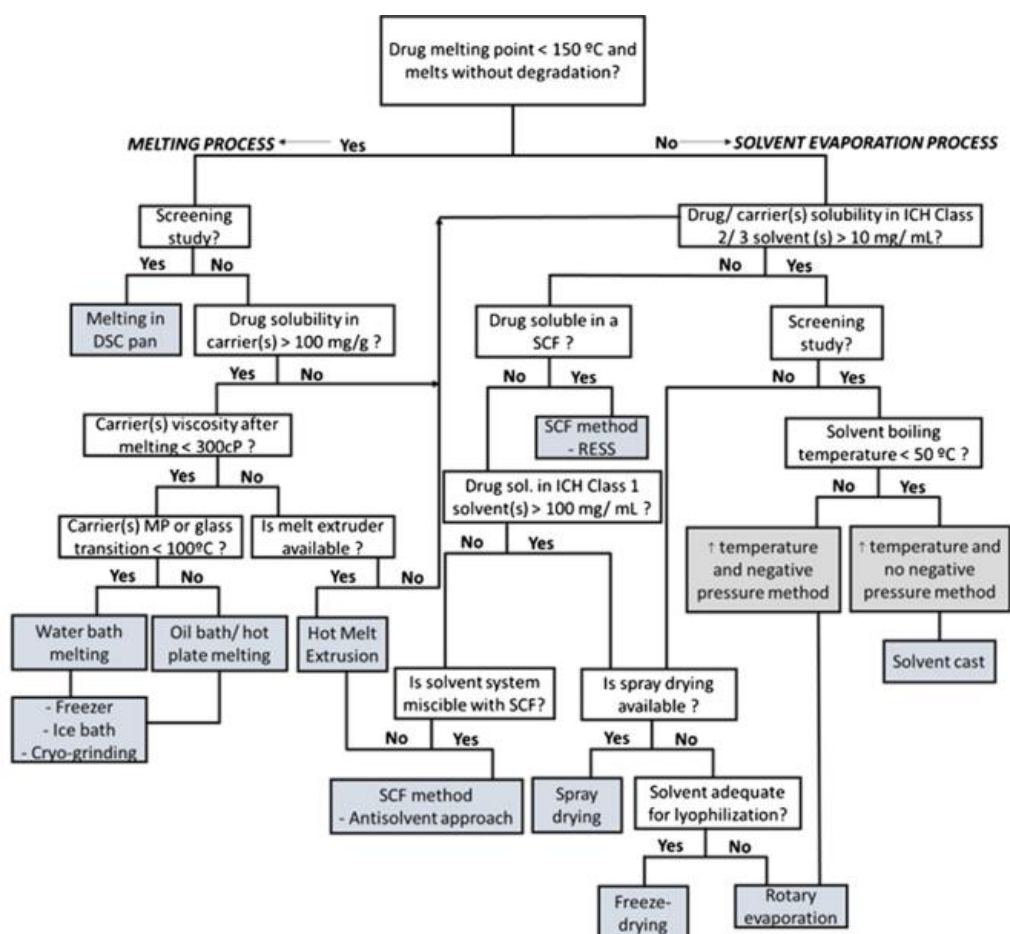


Figure 10 a decision tree for choosing processing method for solid dispersion formulation, from [49]

1.5.5. *In vivo* studies

There are several factors that can limit the bioavailability of a drug, for example dissolution rate, solubility, and permeability. Which formulation strategy increases bioavailability the most depends on which factor is the limiting one. This is why an early *in vivo* study can be beneficial as a tool to determine which limiting factors have to be addressed in a formulation development. Such a study can be performed in rats, or possibly dogs, where the exposure of a drug from a suspension- and a solution formulation is compared. Whether there is a difference between the two formulations and how the exposure differs indicates what the limiting factor is and what formulation strategy can be used to improve the bioavailability (Figure 11).

Thus if the two formulations give equivalent exposure and have high bioavailability, this indicates that the drug is BCS class 1. Similarly, if the formulations are equivalent but have low bioavailability the API may be considered a BSC class 3 drug, limited by permeation or possibly first pass metabolism. If the exposure from solution is much higher than that from suspension this indicates that the bioavailability of the solid API is limited by solubility or dissolution rate. However, for the opposite case, where suspension gives higher exposure than solution, it might indicate chemical instability in the acidic environment of the stomach or precipitation of the drug into a less soluble form [85–88].

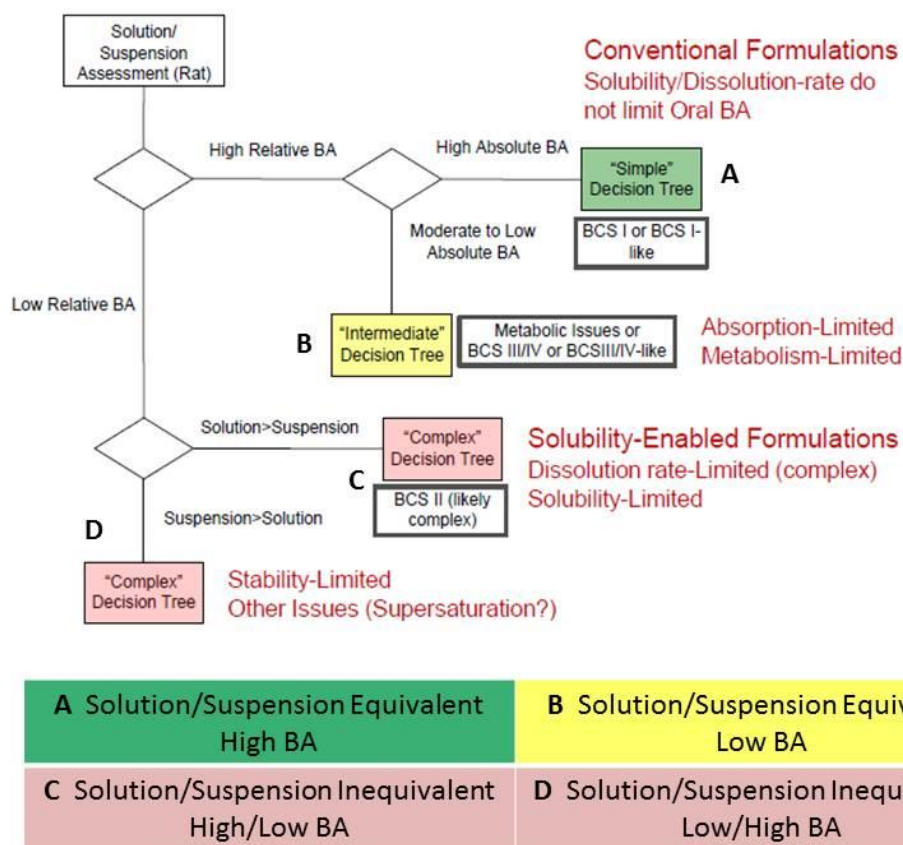


Figure 11 comparison of solution and suspension formulations in vivo can indicate the limiting factor in bioavailability and guide formulation route selection, adapted from [86]

1.5.6. Predictive models

1.5.6.1. Flory Huggins

Mathematical models have been used to predict drug miscibility in polymers. Good miscibility in polymer carriers is pivotal for obtaining sufficient stability for solid dispersions. By using prediction methods, the most likely polymer and or drug loading can be chosen at the start of the formulation development without the need to test several mixtures.

Flory-Huggins (FH) theory proposes an equation for calculating the free energy of mixing of a drug-polymer binary system, Equation 1. The FH interaction parameter χ indicates the interaction between API and polymer and can be estimated experimentally e.g. by melting point depression, recrystallization method and dissolution end point method [89]. Miscibility is achieved when the total free energy of mixing is less than zero [90]. The Flory-Huggins equation, and variations of it, have been widely used to predict polymer-drug miscibility [90–95].

$$\frac{\Delta G_m}{RT} = n_d \ln \phi_d + n_p \ln \phi_p + n_d \phi_p X_{dp}$$

Equation 1 Flory Huggins equation to calculate the free energy of a binary polymer-drug mixing. The following abbreviations are used, ΔG_m is the Gibbs free energy, R is the gas constant, T the absolute temperature, n_d and n_p the number of moles of drug and polymer, respectively, ϕ_d and ϕ_p are the volume fractions of drug and polymer, respectively. X_{dp} Flory-Huggins interaction parameter between drug and polymer.

1.5.6.2. Molecular Dynamics

Molecular dynamics (MD) is the utilisation of molecular mechanics through computational modelling to observe the atomistic behaviour of systems over time. MD approach can potentially give an insight into the molecular interactions and underlying mechanisms in a system. It has therefore increasingly been applied in studies of amorphous solid dispersions, as well as in other fields.

MD has been successfully used to predict the T_g of sucrose in the presence and absence of water [96]. Other applications include investigating and predicting miscibility of API in polymer [97–99].

1.5.6.3. Statistical models

Models are very useful tools in formulation development. A model can give a prediction of an outcome for specific experiments, such as stability or dissolution, thus limiting the need for experimentation and reducing both cost and time spent in development. Building a statistical-based model requires a substantial amount of data and will only be as general as the dataset that was used to create it. However, models can in many cases give accurate predictions and find correlations that are otherwise hard to identify.

Recently Nurzynska and associates [100] successfully built models to predict physical stability of amorphous APIs, based on experimental data of 25 drugs. The best model included measured, calculated and predicted parameters, see Equation 2.

$$\log \text{Stability (days)} = -0.02 T_m - 0.05 H_f + 0.03 T_g + 0.28 G_c - 0.01 \tau + 0.23 \text{HBD} - 0.28 \text{clogP} + 0.17 \text{CHA} + 6.42$$

Equation 2 The abbreviation used in the model are: T_m is the melting temperature in °C, H_f is the enthalpy of fusion in J/g, T_g is the glass transition temperature in °C, G_c is the configurational free energy in kJ/mol, τ is the relaxation time in h, HBD is the number of hydrogen bond donors, clog P is the lipophilicity and CHA is the ratio of carbons to heteroatoms

This is the first model to successfully predict long-term amorphous stability of APIs. This study shows that it is possible to build successful predictive models to help with formulation development. This model could indicate whether an amorphisation process would be feasible for an API in development, based on only predicted, calculated, and easily measured parameters that would be available early in the development process.

Similar models have been built to predict glass forming abilities of APIs and the stability of the amorphous form. These concluded a rule of thumb that APIs with a molecular weight of 300 Da or more are glass formers [46,101]. Other applications of statistical models in drug development include prediction of solid dispersion dissolution [102], drug solubility [103,104] and crystallisability of molecules [105].

1.6. Thesis aim and overview

Despite improvements in drug discovery and development processes, poor solubility continues to be a problem for a substantial portion of NCEs. Several possible routes can be taken to improve solubility of an API. Solid dispersion systems are a good formulation strategy to improve the solubility of poorly soluble compounds but stability issues remain a big hurdle to overcome. Through optimal choice of polymer and manufacturing method, acceptable stability can be achieved. The use of knowledge based support tool in formulation development can decrease the material, time and labour spent on experiments. These can thus accelerate the progress of a NCE through formulation and maximise the chance of finding an optimal formulation. Although much has been done in both fields of solid dispersion and support tools, the literature is still lacking in the following areas

- There is little agreement between individual studies regarding factors governing physical stability of solid dispersions, e.g. T_g of polymer and specific interactions between polymer and API
- Typically, each study only tests a small number of solid dispersion formulations, thus, making general conclusions and assumptions is difficult
- No publicly available knowledge based support tool for solid dispersion formulation is currently available

Therefore, the overall aims of this thesis are

- To investigate the impact of polymer and manufacturing methods on the stability of APIs using a large number of formulations
- To build a predictive statistical model which can be used to aid the formulation development of solid dispersion formulations. The model should be able to rank formulations of different polymer – manufacturing method combinations according to their predicted stability. Such a ranking would give valuable information on starting point in formulation development.

The experimental work is divided into two major chapters. Chapter 2 contains production of solid dispersions and stability measurements. Chapter 3 focuses on building the multiple linear regression models, physicochemical properties of the API and validation of the models.

Chapter 2 – Stability of solid dispersions

2.1. Introduction

With the increasing number of poorly soluble NCEs, the use of, and interest in solid dispersion based formulations has grown. In the years 2011-2016, 101 publications were made on the topic compared to 27 for the eleven previous years (2000-2010) [6]. Although many studies have been devoted to elucidating the most influential parameters related to solid dispersion stability, no clear conclusions have been made. This is in part due to the limited number of APIs studied. Generally, only one or at best a few compounds are investigated in each study which causes difficulties when drawing and validating general conclusions [106–109]. Another problem is that even though there are general guidelines regarding performing stability studies from the ICH and FDA (Table 2), the conditions used and the period tested varies greatly between studies. A temperature range of 2-60 °C and a relative humidity (RH) from 0 % to 100 % has been used in the literature with the period tested ranging from 24 hrs to two years [6,109–115]. Due to these large variations in the design of stability studies, direct comparison of results in the literature is almost impossible. This can also explain why formulations that are found to be stable in one study prove to be unstable in another, where different conditions have been used [6]. Furthermore, varying analytical methods are used to confirm the amorphicity of the API in the solid dispersions. This is especially important when considering low drug loading formulations where the sensitivity of the analytical methods can have a great impact on the stability data. Commonly used techniques to characterise amorphous solid dispersions; detect crystallinity, polymer – drug interactions or confirm amorphicity are X-ray powder diffraction (XRPD) [50,110,116–118], differential scanning calorimetry (DSC) [47,116,117,119,120], polarised light microscopy (PLM) [92,121,122], Raman spectroscopy (RS) [92,123], Fourier-transform infrared spectroscopy (FT- IR) [111,116,124,125], atomic force microscopy (AFM) [126], scanning electron microscopy (SEM) [58,117,127] and nuclear magnetic resonance (NMR) [125].

Table 2 Guidelines for stability studies, the storage conditions and the minimum time period tested, according to ICH [128] and FDA [129]. “*It is up to the applicant to decide whether long term stability studies are performed at 25°C ± 2°C /60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH. **If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition” [128].

Study	Storage condition	Minimum time period covered by data at submission
Long term*	25°C ± 2°C /60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

Despite solid dispersions being the subject of many studies, their formation and physical stability is, as stated, not fully understood [17,130]. However, numerous mechanisms have been proposed, including anti-plasticisation or reduction of molecular mobility, crystallisation inhibition [125,131] and polymer – API interactions such as hydrogen bonding or ionic interactions [132,133].

Specific interactions between the API and the polymer in the solid dispersions, although weak, can immobilise the API. By non-covalently binding to the polymer, the API has, it is proposed, reduced mobility and thus is less likely to find another API molecule to nucleate with and eventually crystallise [40].

A successful production of a solid dispersion contains an underlying assumption of a homogeneity or miscibility of the drug in the polymer on a molecular level [126]. When crystallisation occurs, it is considered a two-step process, nucleation and crystal growth. Crystallisation starts with the formation of a nucleus. This is formed when a critical number of molecules assemble. Subsequently, crystal growth occurs when molecules in the amorphous phase transfer to the surface of the nuclei. Both steps require rearrangement of molecules or diffusion to form aggregates and nuclei or crystal growth. Molecular mobility is therefore a key aspect in the crystallisation of amorphous material [120]. However, crystallisation is a complex process, not only governed by kinetics but also driven by thermodynamic factors. Crystal

growth rate, like nucleation rate, is highly dependent on temperature. It is fastest between the melting temperature and the glass transition temperature, while diminishing at low temperatures. At lower temperatures the thermodynamic driving force for crystallisation is large while at higher temperature molecular mobility causes faster crystal growth. As these have the opposite dependency on temperature the overall maximum growth rate will be between the two as depicted in Figure 12 [122].

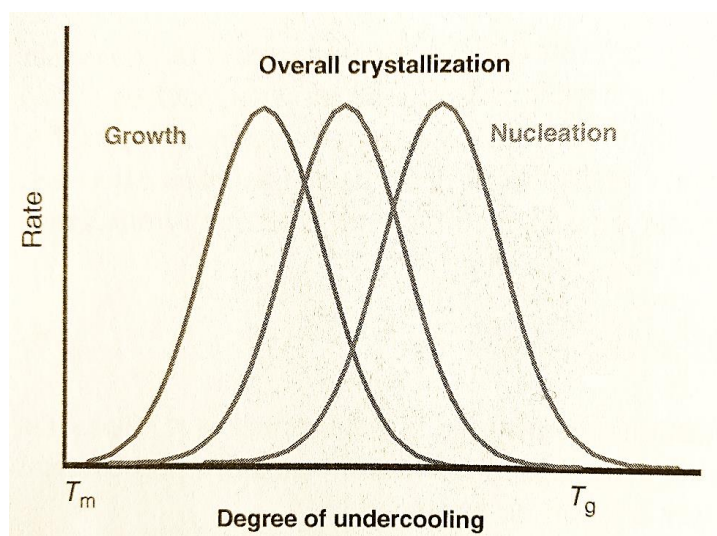


Figure 12 Schematic illustration of the overall crystallisation rate, growth and nucleation depending on temperature, from [122].

Molecular mobility can be divided into global mobility, referred to as α – relaxations, and local mobility, called β –relaxations. The α -mobility is generally associated with larger length scales and arises from the translational and rotational movements of a molecule. In contrast, β -mobility occurs at a short length scale and is a much faster motion [122]. Numerous studies have investigated the relationship between these motions and crystallisation [134,135]. Studies have noted a correlation between high molecular mobility and high crystallisation rates for APIs [136–138]. Molecular mobility decreases rapidly below the T_g and at a temperature of 50°C below the T_g is commonly said negligible (α – mobility). However, crystallisation of glasses has been observed below T_g [100,139–141] and as low as 55°C below T_g [142].

Structural relaxation time and viscosity are both strongly dependant on temperature. When the temperature of an undercooled liquid decreases and approaches the glass transition temperature, the viscosity increases while the system's free volume decreases. This causes the structural relaxation time, the time it takes for a molecule to diffuse a set distance, to increase [120].

Despite difficulties in the prediction of amorphous stability or crystallisation based on molecular mobility measurements, it is clear that factors that increase molecular mobility will increase the chance of crystallisation. Numerous factors can affect molecular mobility but temperature and moisture content are the two with the greatest practical importance [122]. Water is a well-known plasticiser, lowering the T_g of solid dispersions. Water absorption due to storage at high relative humidity conditions has been shown to lower T_g and increase crystallisation rate in solid dispersions [53,143]. Polymers, in contrast, work as anti-plasticisers, increasing the T_g of the solid dispersion compared to the API, and thus decreasing the molecular mobility [120].

2.2. Drug selection

Choosing a diverse selection of APIs for the studies here was pivotal. In order to be able to draw general conclusions about stability of solid dispersions and the effect of the APIs properties on this, the dataset must be as diverse as possible. Furthermore, as the experimental data was used to build a statistical model any limitations of the dataset restrain the application of the model. As the sample size was relatively small (although large compared to any other published study), ten drugs, some limitations to the diversity were, however, necessary in order to have a possibility of finding a correlation between API physicochemical properties and the stability of the solid dispersions. When deciding the constraints for choosing the APIs three parameters were considered. Firstly, only poorly water soluble ($\log S < -4$) neutral APIs, defined as having $pK_{a, \text{acidic}} > 8.5$ and $pK_{a, \text{basic}} < 4.5$, with a molecular weight less than 800 g/mol were considered. These restrictions were based on previous work which had produced statistical models that could

successfully predict the stability of amorphous APIs [100,144]. Secondly, physicochemical properties that are likely to influence stability or manufacturability were considered to ensure APIs with more extreme values, which might be more prone to crystallise, were represented as well as more stable APIs. The parameters and their values are shown in Table 3. These properties included melting point (mp) which can be important for HME in particular and the number of rotatable bonds (nRot) and rings (nRing), which has been found to correlate with stability [47,56]. Furthermore, H-bond donors and acceptors (HBD, HBA) and lipophilicity (logP) were included as these can be important when considering possible interactions with the polymer.

Finally, the glass forming ability of the APIs was considered. Drugs can be classed into three categories depending on their ability to form amorphous glass and on the stability of the glass state. The three classes are non-glass formers (class I), non-stable glass formers (class II) and stable glass formers (class III) [43,46]. The ten drugs chosen (see chemical structures in Figure 13) were equally divided between class II and III. No APIs from class I were included, as these are classified as non-glass forming it was deemed they would be unlikely to be stable in amorphous solid dispersions. The difference between class II and class III would be expected to influence formulation stability. Class III drugs are stable glass formers and therefore, their stability should be less influenced by the formulation. The ten APIs chosen are shown in Figure 13 and listed in Table 3, they were divided between the two GFA classes as follows, class II: Aprepitant, Celecoxib, Estradiol, Nifedipine and Orlistat, class III APIs were Felodipine, Fenofibrate, Itraconazole, Probuco and Ritonavir.

Table 3 Calculated and predicted parameters that were used to ensure diversity of the API sample set. The following abbreviations are used: MW - molecular weight, HBD - H-bond donors, HBA - H-bond acceptors, $pK_{a, \text{basic}}$ – predicted strongest basic pK_a , $pK_{a, \text{acidic}}$ – predicted strongest acidic pK_a , nRot - rotatable bonds, nRing - Ring count

	<i>Nifedipine</i>	<i>Felodipine</i>	<i>Itraconazole</i>	<i>Probuco</i>	<i>Ritonavir</i>	<i>Fenofibrate</i>	<i>Celecoxib</i>	<i>Estradiol</i>	<i>Orlistat</i>	<i>Aprepitant</i>
<i>GFA</i>	II	III	III	III	III	III	II	II	II	II
<i>MW</i>	346.3	384.3	705.6	519.8	721.0	360.8	381.4	272.4	495.8	534.4
$pK_{a, \text{basic}}$	-6.63	-6.64	3.92	-5.08	2.84	-4.93	0.08	-0.88	-1.44	3.74
$pK_{a, \text{acidic}}$	19.4	19.5		10.3	13.7		10.7	10.3	12.7	7.6
<i>HBA</i>	5	3	9	2	6	3	3	2	3	6
<i>HBD</i>	1	1	0	2	4	0	1	2	1	2
<i>logS₀</i> (M)	-3	-4	-9	-11	-8	-6	-6	-4	-11	-6
<i>mp</i> (°C)	173	145	166	125	121	81	158	152	44	255
<i>logP</i>	1.82	3.44	7.31	10.57	5.22	5.28	4.01	3.75	8.11	4.52
<i>nRing</i>	2	2	7	2	4	2	3	4	1	4
<i>nRot</i>	6	6	11	8	18	7	4	0	23	8

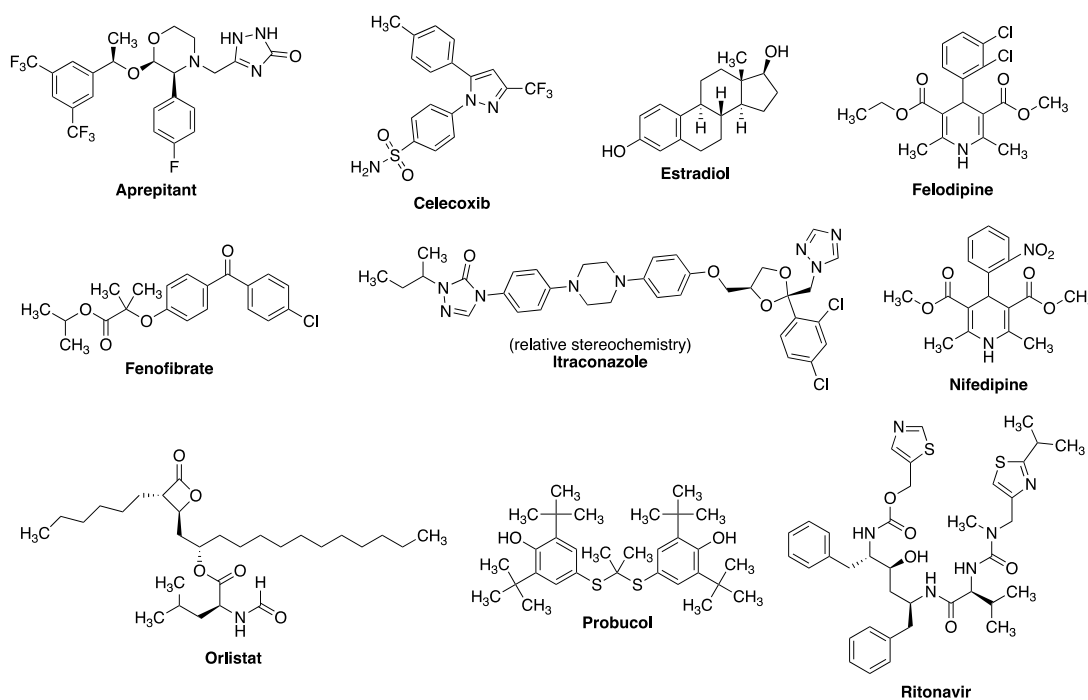


Figure 13 Chemical structure of the ten APIs used in this study.

2.3. Polymer selection

The dataset used in this study should represent the general practice in solid dispersions. Furthermore, the use of the statistical models will be limited to the polymers they are based on. Therefore, it was important to choose the polymers to best represent those currently most used in solid dispersions. A research of marketed solid dispersions in oral dosage forms showed that such formulations are mostly made with one of three types of polymers, cellulose based polymers (HPMC, HPC or HPMC-AS), polyvinyl polymers (PVP and PVP-VA) or PEG (see Table 4) [38,41,145]. The three polymers used in this project were therefore chosen to represent these. The chosen polymers were HPMCAS, PVP-VA and Soluplus™.

Table 4. Summary of marketed solid dispersions in oral dosage forms, including the brand name, the APIs they contain and the polymer carriers used [38,41,145]. The following abbreviations are used: PEG - polyethylene glycol, PVP – polyvinylpyrrolidone, HPMC - hydroxypropyl methylcellulose, HPMCAS - hydroxypropyl methylcellulose acetate succinate, HPC - hydroxypropyl cellulose.

<i>Brand name</i>	<i>API</i>	<i>Polymer carrier</i>
<i>Adalat SL</i>	Nifedipine	PEG
<i>Afeditab CR</i>	Nifedipine	Poloxamer-PVP
<i>Certican</i>	Everolimus	HPMC
<i>Cesamet</i>	Nabilone	PVP
<i>Crestor</i>	Rovustatin	HPMC
<i>Envarsus</i>	Tacrolimus	HPMC
<i>Fenoglide</i>	Fenofibrate	PEG
<i>Gris-PEG</i>	Griseofulvin	PEG
<i>Incivo</i>	Telaprevir	HPMCAS
<i>Intelence</i>	Etravirine	HPMC
<i>Isoptin SR-E</i>	Verapamil	HPC/HPMC
<i>Kaletra</i>	Lopinavir/Ritonavir	PVP-VA
<i>Kalydeco</i>	Ivacaftor	HPMCAS
<i>Lopinavir/Ritonavir</i>	Lopinavir /Ritonavir	HPMCAS
<i>Mylan</i>		
<i>Modigraf</i>	Tacrolimus	HPMC
<i>Nivadil</i>	Nivaldipine	HPMC
<i>Noxafil</i>	Posaconazole	HPMCAS
<i>Orkambi</i>	Lumacaftor / Ivacaftor	HPMCAS
<i>Prograf</i>	Tacrolimus	HPMC
<i>Rezulin</i>	Troglitazone	HPMC
<i>Sporanox</i>	Itraconazole	HPMC
<i>Viekirax</i>	Ombitasvir/Paritaprevir/Ritonavir	PVP-VA
<i>Votubia</i>	Everolimus	HPMC
<i>Zelboraf</i>	Vemurafenib	HPMCAS

2.3.1. Polyvinyl caprolactam - polyvinyl acetate - polyethylene glycol

Polyvinyl caprolactam (PVC) – polyvinyl acetate (PVA) – polyethylene glycol (PEG) or Soluplus™ (SOL) as it is commercially known, is a relatively new polymer, marketed from 2009 by BASF. Soluplus™ was specifically developed for use in solid dispersions, mainly hot melt extrusion, but has also been used for other purposes such as in granulation and to form micelles [146–148]. It is a graft polymer made out of three sections, two of which are lipophilic, the PVC and PVA, while the third, PEG, is hydrophilic, see chemical structure in Figure 14. This amphiphilic nature of the polymer enables it to stabilize hydrophobic drugs while still being very water soluble. It also causes it to form micelles in solution and thus further solubilising the drug. Soluplus™ is freely soluble in both aqueous solutions and organic solvents [61,106,149,150]. It absorbs around 10% wt water at 70% RH (unpublished data, BASF). Due to its low glass transition temperature (T_g) of 70°C it has mainly been used in solid dispersions processed with hot melt extrusion but a few studies have shown its use in spray-drying. Soluplus™ is the only polymer used here, not currently used in a marketed formulation.

2.3.2. Hydroxyl propyl methyl cellulose acetate succinate

Hydroxyl propyl methyl cellulose acetate succinate (HPMCAS) is a synthetic polymer derived from cellulose, Figure 14. It was first marketed by Shin-Etsu as an enteric coating polymer but has since been used for several purposes amongst other as controlled release or sustained release agent, film coating polymer and as a carrier in solid dispersions, using both hot melt extrusion and spray-drying [151]. It is now a commonly used polymer in solid dispersion, partly due to the high T_g of 122°C, and is used as carrier polymer in several marketed formulations (Table 4). It is available in three substitution levels of acetyl substituents, L, M and H, see Table 5 [152,153]. HPMCAS is insoluble in water in the un-ionised form (< pH 5) thus insoluble in gastric fluid but swells and rapidly dissolves at more neutral pH, in the upper small intestine and is predominantly colloidal at intestinal pH (6.0 - 7.5). The amphiphilic nature of the polymer enables it to stabilise hydrophobic APIs while still sustaining stability in aqueous solution. This relative hydrophobicity of

HPMCAS means it is less hygroscopic than other water-soluble polymers. HPMCAS absorbs approximately 5 - 6 % wt water when stored at 75 % RH compared to 23 % and 10 % wt water for PVP and HPMC respectively [81,150].

Table 5 The substitution range and T_g of the three grades of HPMCAS [152]

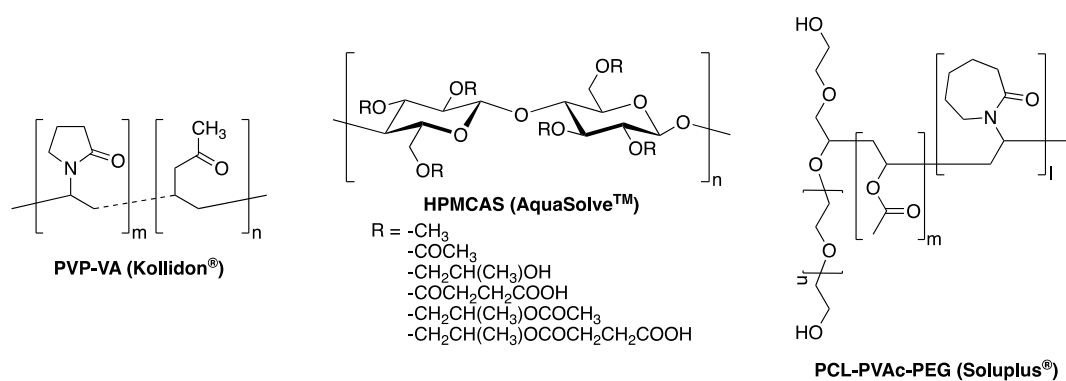
	<i>L grade</i>	<i>M grade</i>	<i>H grade</i>
<i>Acetyl content</i>	5 - 9 %	7 - 11 %	10 – 14 %
<i>Succionyl content</i>	14 – 18 %	10 – 14 %	4 – 8 %
<i>Methoxyl content</i>	20 – 24 %	21 – 25 %	22 – 26 %
<i>Hydroxypropoxy content</i>	5 - 9 %	5 – 9 %	6 – 10 %
T_g	119 °C	120 °C	122 °C

2.3.3. Vinylpyrrolidone – vinyl acetate

Vinylpyrrolidone – vinyl acetate or copovidone (PVP-VA), (chemical structure in Figure 14) is produced by free radical polymerisation of vinyl pyrrolidone and vinyl acetate in a six to four ratio. PVP-VA is a widely used pharmaceutical excipient and is regarded generally as non-toxic. It is a versatile excipient that has many purposes in drug formulations, such as in coating, granulation, as a binder and as a polymer carrier in solid dispersions. PVP-VA is already used as a carrier in a marketed solid dispersion formulation, just as HPMCAS (see Table 4). PVP-VA can be used both for immediate release and for sustained or controlled release when used in combination with other materials [151,154]. PVP-VA has a moderate T_g of 103°C. It is freely soluble in water and polar organic solvents and quite hygroscopic, with a water uptake of approximately 10 % at 50 % RH [151,153].

A comparison of the three polymers shows the diversity of the dataset. An overview of the physicochemical properties is summarised in Figure 14. These properties cover most of the important factors for polymer carriers. A range of T_g from low (SOL) to moderately high (HPMCAS) accounts for the effect of polymer T_g on stability of solid dispersions as well as the manufacturability, mainly via HME. The polymers also have a range of available H-bond donors and acceptors, which

could possibly introduce stabilising H-bonds with APIs, from zero HBD for PVP-VA and SOL to 10-28 HBA for HPMCAS. Both SOL and PVP-VA are neutral polymers, whereby water solubility is unaffected by pH. HPMCAS in contrast is acidic due to its acidic side chains and is only water soluble at a pH above 6.5. Furthermore, HPMCAS and SOL are amphiphilic, which should allow them to more easily stabilise hydrophobic APIs compared to PVP-VA, which is hydrophilic. The water uptake also differs according to the hydrophilicity, PVP-VA absorbs 10% wt water at 50 % RH and SOL 10% at 70% RH while only 5-6% wt water is absorbed by HPMCAS at a higher RH of 75 %.



	PVP-VA	HPMCAS	Soluplus
T_g (°C)	103	122	70
Weight (Da)	45.000 – 70.000	55.000 – 90.000	90.000 - 140.000
HBD	0	0 - 6	0
HBA	2	10 – 28	3
Water uptake (wt %)	10 at 50 % RH	5-6 at 75% RH	10 at 70% RH
pH range where soluble	1 - 10	>6.5	1 - 10

Figure 14 Chemical structure of the three polymers used in this study, PVP-VA, HPMCAS and Soluplus and overview of the polymer properties. The number of HBD and HBA are per unit of monomer. Abbreviations used, HBA – H bond acceptor, HBD – H bond donor. The range of HBD and HBA for HPMCAS depends on the grade of HPMCAS, that is L, M or H [150,152,155].

2.4. Materials and Methods

Ten poorly soluble, neutral, chemically diverse APIs: nifedipine (1), felodipine (2), itraconazole (3), probucol (4), ritonavir (5), fenofibrate (6), celecoxib (7), estradiol (8), orlistat (9), and aprepitant (10), see Figure 13, were purchased from Sequoia Research Products Ltd. The three polymers were generous gifts from their commercial suppliers: Soluplus (SOL) and Kollidon VA64 (PVP-VA) from BASF and AquaSolve (HPMCAS-HG)) from Ashland (Figure 14). The salts NaCl and K₂CO₃ were acquired from Sigma Aldrich while the solvents CH₃OH and CH₂Cl₂ were purchased from Fischer. Molecular sieve 3Å and 4Å was used to keep relative humidity close to 0% and remove possible residual solvents. Binary solid dispersions were made for each combination of polymer and drug. Each combination was produced twice, once using hot melt extrusion (HME) and once by spray-drying. In order to make all findings as industry relevant as possible, drug loadings were kept at a commonly achievable 10% and all formulations were produced in ‘lab-scale’ bulk (around 10g). Each formulation was then subjected to a stability test and characterised at each time point. The following abbreviations of formulations are used as follows with numbers one to ten for the APIs as listed above. For the polymers S, P and H for SOL, PVP-VA, and HPMCAS, respectively. Hot melt extrusion (h) and spray-drying (s). Thus *e.g.* spray-dried ritonavir – PVP-VA is abbreviated 5Ps.

2.4.1. Hot melt extrusion

A HAAKE minilab II micro compounder (Thermo Fisher Scientific) was used for the production of the HME solid dispersions. All formulations were made with 10% w/w drug loading in the polymer. In order to keep the number of variables as low as possible all solid dispersions were produced using the same method and no optimisation was performed for any formulation. The MiniLab required the following conditions, 4 bar compressed air, Nitrogen 3L/min at a pressure of 1bar. Polymer and API were weighed into two scintillation vials 4.95g and 550mg respectively into each. Once the MiniLab was at the set temperature (160 °C) and had been calibrated the contents of one vial was poured into the feeder, a small amount at a time. The timer was started once the full contents of the vial had been

fed into the machine. The twin screws were co-rotated at 50 r.p.m. and the mixture was circulated for 15 min before being extruded (Figure 15). The process was then repeated for the second vial. The same method was used for all formulations except those containing orlistat, which was not circulated but extruded immediately because of its low melting point (44 °C). Once extruded, the formulation was placed into closed, amber HPLC vials and stored at 5 °C / 0% RH until the start of the stability study.

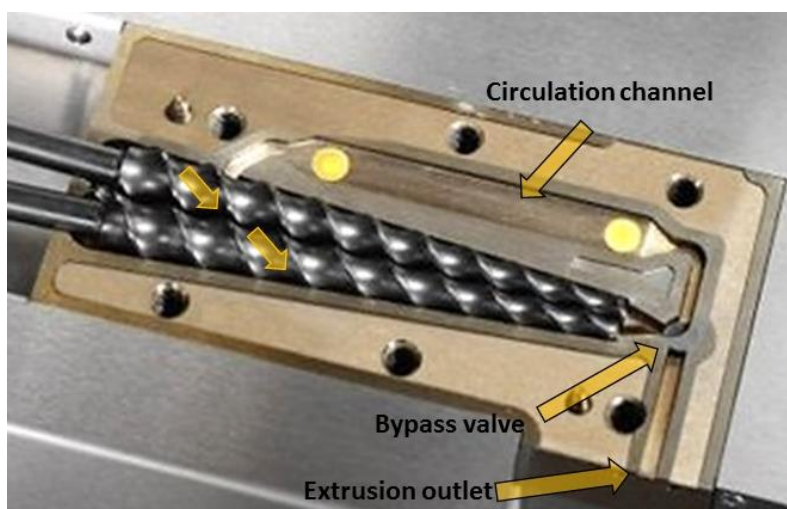


Figure 15. The configuration of the HAAKE minilab II. The two screws co-rotate, kneading and mixing the polymer - API blend together as it is melted. The whole element is heated and the mixture circulated until the bypass valve is turned and the solid dispersion is extruded.

2.4.2. Spray-Drying

As with the HME formulations, to keep the number of variables at a minimum, the same method was used to produce all formulations. All the spray-dried solutions were 10%w/v and all the APIs apart from Itraconazole were dissolved in methanol. Itraconazole was spray-dried from a dichloromethane solution as it was not soluble in methanol. Spray-drying was performed using a Büchi B290 instrument. For all runs the following were kept constant, approximately 3 bar Nitrogen and 90-100% airflow. The inlet temperature was kept between 76°C and 90°C in order to keep the outlet temperature constant at 48-52°C. The flow rate was calculated from the run time and was 4-9ml/min for all runs.

The API and polymer were weighed (1.4g and 12.6g) and the powder mixture transferred into a glass bottle. Roughly 100ml of solvent was poured into the bottle and a magnetic stirrer used to vigorously stir the solution until all the powder was dissolved. The solution was then poured into a measuring cylinder and solvent filled up to 140ml. The solution was then transferred back into the glass bottle and stirred vigorously until it was spray-dried. The spray-drier was warmed up to the desired temperature and the solvent was pumped through until the inlet and outlet temperature had reached the set temperature. The tube was then transferred from the solvent into the formulation solution and the timer started. The inlet temperature, aspiration and pump flow were adjusted if needed to keep the outlet temperature constant at around 50°C. When the solution was finished the timer was stopped and the solvent pumped through the system again. The flow rate and yield was calculated for each run. The powder was collected into a closed, plastic vial and stored in a desiccator in a cold room at 5°C/0%RH until stability study was started.

2.4.3. Stability study

A six-month stability study was conducted on all produced solid dispersions at accelerated conditions, 40 °C / 75% relative humidity (RH), in accordance with ICH guidelines [128]. The samples were kept in amber glass HPLC vials, open containers. Furthermore, all formulations were also kept at 5 °C / 0 % RH and 25 °C/43 % RH. This was done in case all the formulations would prove to be very unstable at the most accelerated conditions. The temperature and humidity was monitored with data-loggers, Testo H-174, that record the conditions every 70 minutes. Roughly 120-200mg of formulation was weighed into amber HPLC vials. For each formulation there were 21 HPLC vials, seven for each condition. The lid was pierced with a large gauge needle, which allowed moisture to flow between the desiccator and into the vial. The amorphicity of the formulations was confirmed at the start of the stability study. Subsequently the solid dispersions were analysed at 12 h, 7 d, 1 month, 2 months, 4 months and 6 months. For those solid dispersions that were still amorphous after 6 months, a 1-year time point was added. At each of these time points the samples were examined for signs of crystallinity using polarised light microscopy (PLM) as a sensitive method to detect onset of

crystallisation. Any formulations with signs of crystallinity by PLM were then further characterised using differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). Before analysing the samples at each time point the formulation was ground in a mortar, if necessary, producing small glass-like pieces appropriate for characterisation with PLM, DSC, and XRPD.

Time constraints prevented the stability of each formulation to be started at the day of production. The ready-made formulations were therefore stored at 5°C and 0% RH until the first stability point. The stability study was started once the samples were put in their respective storing condition, 20°C/43% or 40°C/75% RH.

GraphPad Prism 7.03 was used analyse and plot data.

2.4.4. Differential scanning calorimetry

A Q2000 (TA instruments) was used for the DSC measurements of all samples, using Tzero pans (TA instruments) and pierced lids. The instrument was calibrated against indium. Approximately 5 mg of sample was weighed into each pan. Each sample was run in duplicate. All solid dispersions, except with orlistat, were run as a cycle where the first ramp was used to remove water and solvent from the formulation. The second time the sample was ramped up to 20 °C above the melting point of the drug. Both up and down ramps were performed at 10 °C/min. As orlistat has a melting point of 44 °C it was not feasible to go above 100°C in order to remove water before running the sample as this might have affected the solid dispersion. It was therefore run as a single ramp. The results were analysed using the TA Universal Analysis programme to determine T_g and melting points (mp) and plotted with GraphPad Prism.

2.4.5. X-ray powder diffraction

A Bruker D8 Advance instrument using a Cu K α 1 radiation source and equipped with an incident beam monochromator was used for the XRPD measurements. The instrument was calibrated against corundum. All samples were analysed with spinning at a current of 40 mA and a voltage of 40 kV using standard scanning parameters, *i.e.* from 2 to 40° (2 θ) using a step size of 0.02° and a time per step of 1 s. A zero-background holder was used for the measurements of samples of approximately 3-5 mg. The program DIFFRAC.SUIT EVA from Bruker was used to analyse and plot the data.

2.4.6. Polarised light microscopy

An Olympus BX51 optical microscope fitted with polarising filters and equipped with an Olympus DP71 camera was used visually to check for presence of any crystalline material. A small amount of powder (spray-dried) or ground glass (HME) was placed on a microscope slide with a glass cover slip. The samples were examined using both cross-polarised and non-polarised light. Images of identical frames were recorded for each sample under both polarised and non-polarised light. Cell[^]F imaging system from Olympus was used to acquire images of the samples.

Crystals were defined as bright white or coloured spots under polarised light. In each case it was ascertained that spots in the PLM images displayed a distinctive pattern that was not due to any physical defects of the surfaces of the examined samples, such as cracks and sharp edges or curved edges of a sphere (spray-dried particles). The bright spots were a part of the sample piece (HME glass or spray-dried particle), on the surface or inside. The bright spots had similar geometric shape, such as star-like or rectangular, and colour (white or multicolour). Examples can be seen in Figure 17, Figure 19 and Figure 21. Even though the onset of crystallisation was the main outcome of this study, a crystallised formulation was followed up at each time point after onset to confirm that further crystallisation had occurred and that the first onset point was not an artefact of measurement.

2.5. Results

Due to limit of detection (LOD) of XRPD and DSC, PLM was used as the main method of detecting the onset of crystallisation. In many cases, even when an abundance of crystals was observed by PLM, no crystallinity could be detected with either XRPD or DSC (Figure 16). Only one crystal needed to be observed in a sample for that formulation to be classed as crystalline. Therefore, it was possible to detect the onset of crystallisation very early.

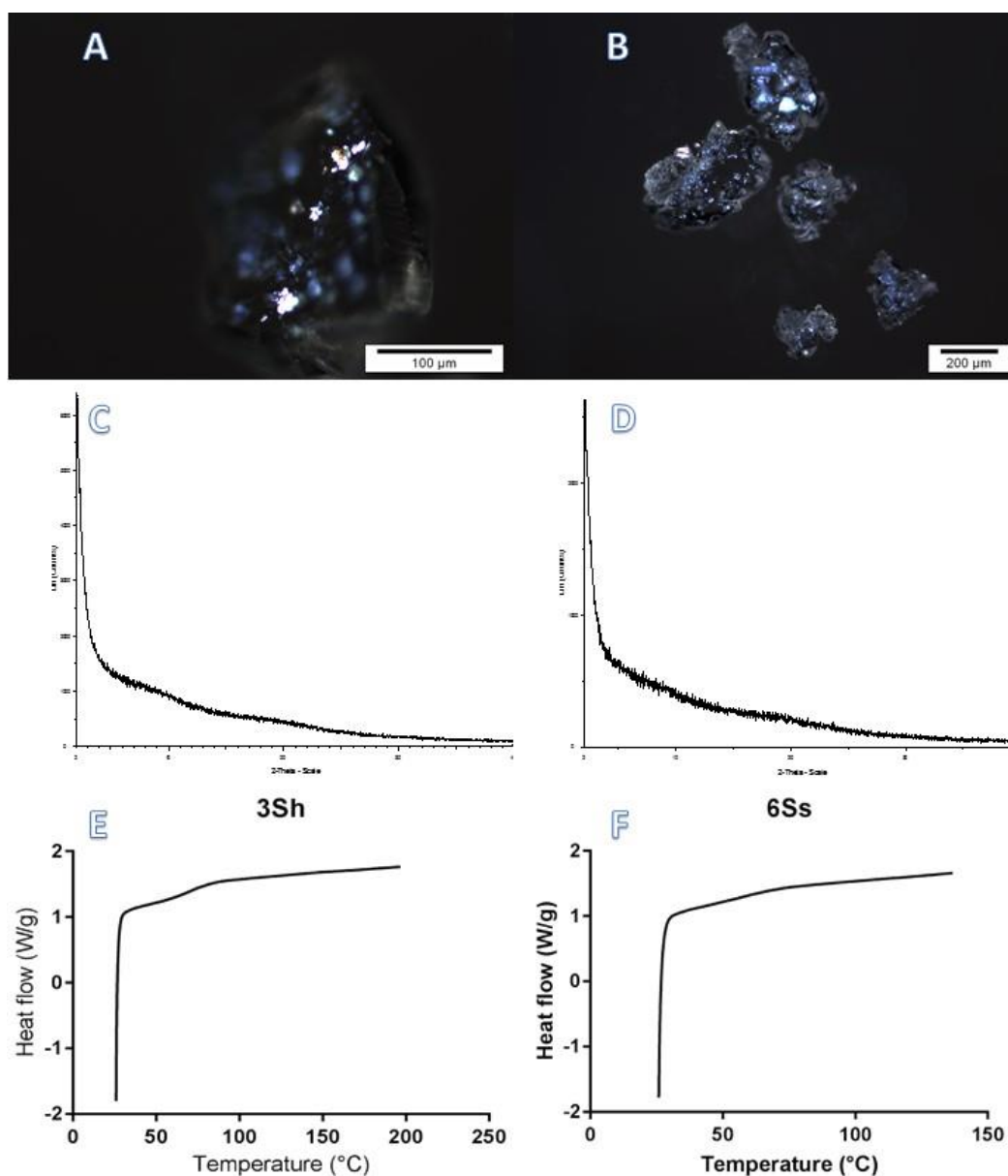


Figure 16 A) 3Sh with clearly visible crystals under polarised light, C) and D) no melting peak is visible under DSC nor any peaks in XRPD. B) 6Ss with abundance of crystals visible, again no melting peak by DSC nor peaks indicating crystallinity in XRPD, D) and F).

The stability study at the least accelerated conditions of 5 °C/0 % RH was discontinued when the stability of the formulations at 40 °C/75 % RH proved to be sufficiently long so that it was possible to distinguish between formulations. Furthermore, only negligible signs of crystallinity were observed at the less extreme conditions of 25 °C/43 % RH, even at the end of the stability study at six months and one year (see Figure 17). While an abundance of crystals were observed in all samples at the most accelerated conditions, no signs of crystallinity were observed at the intermediate conditions. Furthermore, when comparing the two spray-dried samples, panel A and B, the accelerated conditions have caused transformation of the sample and an abundance of crystals while single sphere particles are still observed in the well dispersed sample at the intermediate conditions.

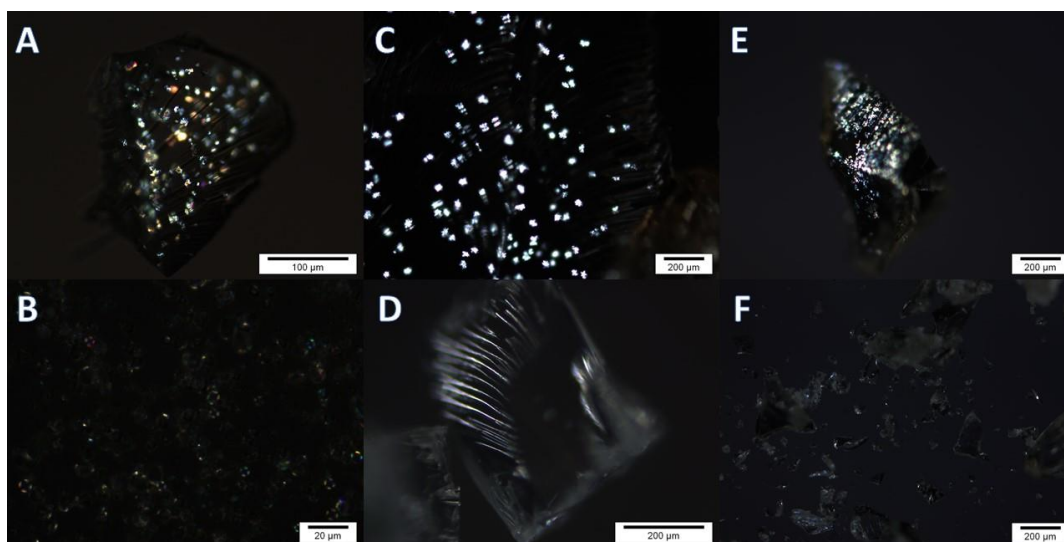


Figure 17 shows the crystallinity observed in formulations stored at the accelerated conditions, 40 °C/75 %RH (A, C, E) and corresponding formulations at the same time point but stored at 25 °C/43 % RH (B, D, F). The formulations shown are A) and B) 4Ps at 6 months, C) and D) are 8Sh at 12 months and E) and F) show 3Sh at 6 months.

2.5.1. Stability of formulations

For each API-polymer mixture the HME solid dispersion appeared as a clear glass after extrusion, an indication of amorphicity, except 10Hh, which was cloudy. At the start of the stability assessment all the clear glass HME formulations and all the spray-dried formulations for all APIs were confirmed to be amorphous, while the cloudy 10Hh formulations were confirmed to contain crystals.

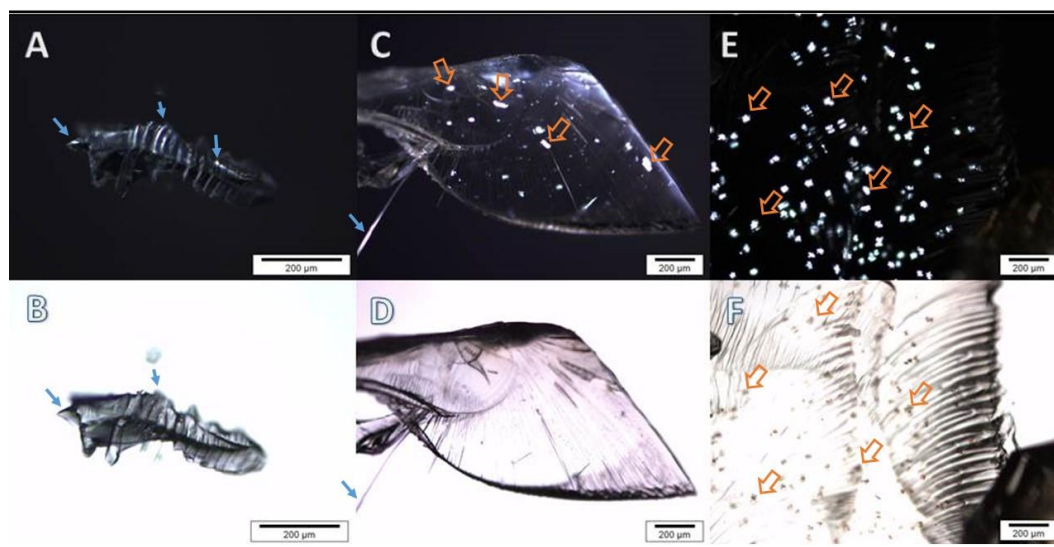


Figure 18. Pieces of HME formulations under polarised light (A, C and E) and non-polarised light (B, D and F). (A) 8Ph sharp edges (blue, thin arrows) are visible under polarised light, the visible lines are confirmed as part of the glass rather than crystals (B). Panels C and E show visible crystals (orange, thick arrows) under polarised light, 9Hh and 8Sh respectively. No sign of crystals can be seen under normal light in D but crystals are clearly observed in F.

At the start of the stability study the spray-dried solid dispersions were dispensed into the storage vials as dry dispersible powders, but after a period stored at 40 °C / 75% RH most of the powders had converted to solid glasses, similar in appearance to the HME glass. In most cases signs of crystallisation were not seen until after the transformation of the powder to the glass. How soon this transformation was observed depended on both the polymers and the APIs as some solid dispersions were more resistant. An example of this transformation can be seen in Figure 19 where the first image shows a well-dispersed powder; panels C and D shows a piece where signs of merged particles can be seen but in the last image no signs of previous particles are observed. This transformation to glass was not observed

under less accelerated conditions and not for any of the HPMCAS spray-dried formulations.

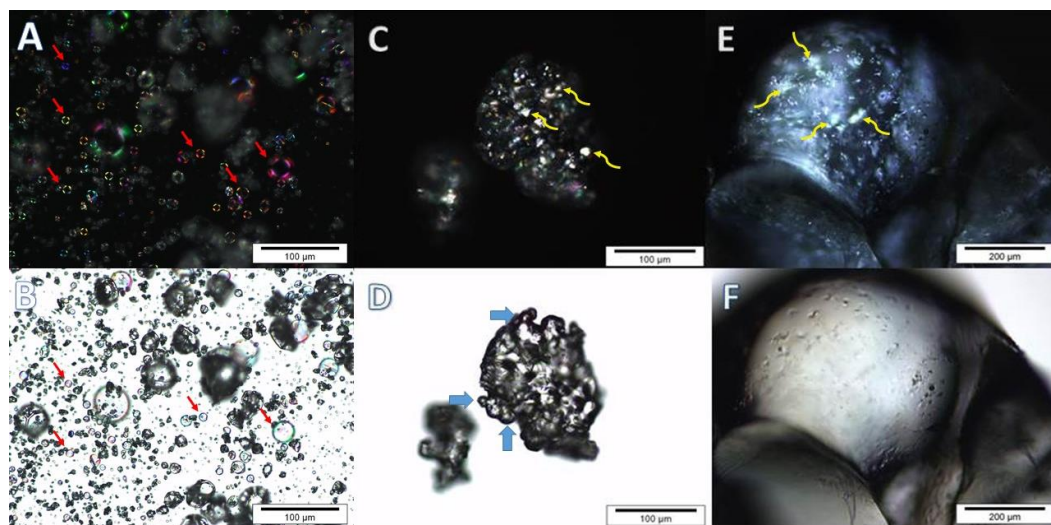


Figure 19. Panels A, C, and E show solid dispersions under cross-polarised light while panels B, D, and F show the same samples imaged under normal light. Panels A and B show a well-dispersed powder, 6Hs, where typical bubble-like particles (red, thin arrows) can be observed. Panels C and D show a piece of 6Ps where the powder has transformed into glass. It is possible to still see signs of the original particles that have merged (blue, thick arrows). Panels E and F show a large piece of 6Ss formulation. Here the transformation into glass is more complete than in (D) as no signs of previous particles can be observed. Crystallisation can be observed (yellow, curvy arrows) in C and E.

The onset of crystallisation was noted for each formulation. For those solid dispersions, where no signs of crystals could be found after 6 months at 40 °C / 75 % RH a 1-year time point was added. Once the onset of crystallisation had been noted observations of the samples were continued in order to confirm the crystallinity of the sample. Specifically in those cases where the first signs of crystals were a single bright spot in the PLM such as was the case in formulation 6Ph at 2 months, see panel F in Figure 20. The extent of progression of crystallisation between time points varied notably. In two months, from 8 weeks to 16 weeks the amount of crystals observed in 6Ph did not change significantly. This was, however, not the case for 6Ps where in the same time frame the samples went from showing a large amount of bright coloured spots in panel J to being covered in bright crystals in panel K.

In Figure 21 the stability, *i.e.* the last time point where no signs of crystals were observed, is summarised for each API–polymer combination. For example, if crystals were first observed at 4 weeks, crystals must have started growing between time points 1 week and 4 weeks. Thus the last data point of confirmed amorphicity is 1 week and that is the noted stability.

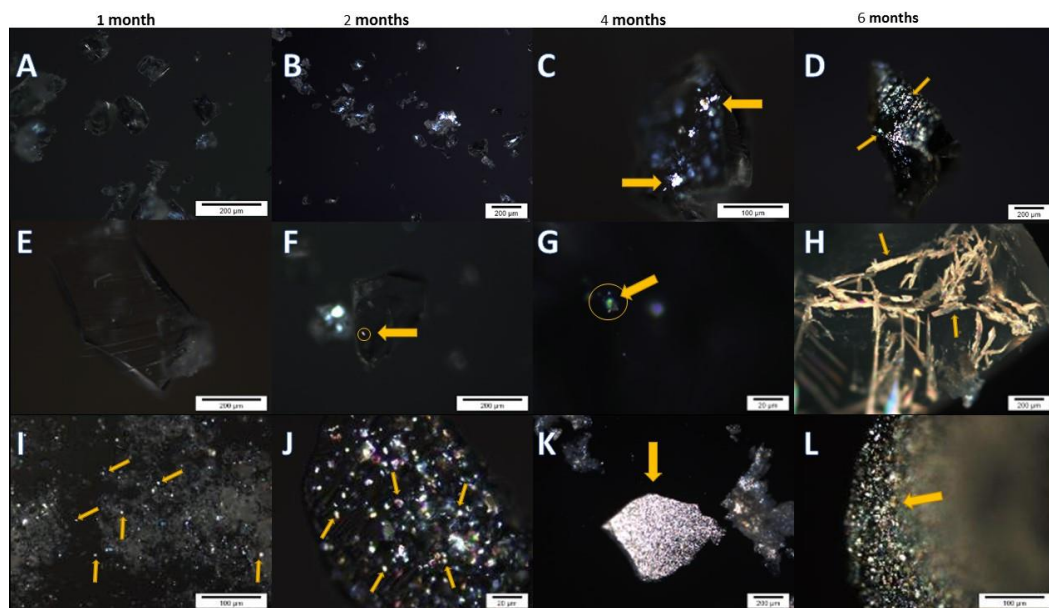
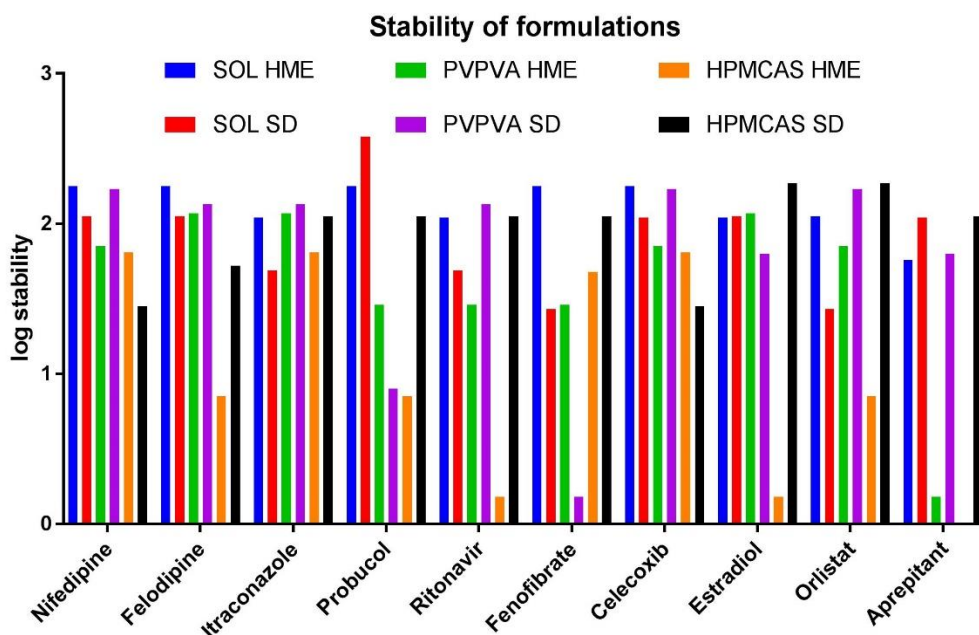


Figure 20 shows the progression of crystals over time. A-D is 3Sh, E-H is 6Ph and I-L is 6Ps. Panel A, E and I show the sample at 4 weeks, panels B, F and J at 8 weeks, 16 weeks is represented in panels C, G and K and finally 24 weeks in panels D, H and L. The onset of crystallisation was determined to be 4 months for 3Sh, 2 months for 6Ph and 1 week for 6Ps (image not shown). Crystals are highlighted with yellow arrows.

An overview of the difference in stability is provided in Figure 21. Significant differences in stability between the polymers can be observed for some APIs. As an example, the last amorphous time point for felodipine was observed at 1 week in the HPMCAS HME formulation, at 16 weeks when formulated with PVP-VA by HME and at 24 weeks with SOL by HME. A similar example can be found in the spray-dried formulations, probucol was only stable for 1 week in PVP-VA, for 111 days in HPMCAS and was still amorphous at the one-year point (379 days) in SOL. Some similarities are also observed between the polymers, aprepitant seems to be the least stable of all the drugs by HME regardless of the polymer used, although the onset of crystallisation time varies from zero to 16 weeks.

Additionally, differences were observed between the two methods, spray-dried formulations seem to be more stable for PVP-VA and HPMCAS while the opposite is true for SOL formulations.



		Orlistat	Fenofibrate	Ritonavir	Probutol	Felodipine	Estradiol	Celecoxib	Itraconazole	Nifedipine	Aprepitant
SOL	HME	112	176	109	176	176	109	176	56	176	57
	SD	26	26	48	379	110	110	109	48	110	109
PVPVA	HME	70	28	28	28	116	116	70	116	70	0.5
	SD	170	0.5	133	7	133	62	170	133	170	62
HPMCAS	HME	6	47	0.5	6	6	0.5	63	63	63	0
	SD	185	111	111	111	52	185	27	111	27	111

Figure 21 shows the measured stability (last time point where no crystals were observed) in days, in the table on the bottom, for each formulation. Graph shows the plotted data as $\log(\text{Stability})$.

2.6. Discussion

2.6.1. Sensitivity of analytical methods

Most studies considering the stability of solid dispersions rely on DSC and XRPD for detecting crystals, not PLM [38,109,119,156,157]. However, it has been observed in this study that specifically at low drug loading these two common methods lack the sensitivity to detect onset of crystallinity accurately. Here PLM has therefore been used as a primary method of detection as it is known to be a sensitive and rapid method to detect small amounts of crystalline material in amorphous matrixes [121,122,158–160]. The LOD of crystallinity in samples with XRPD is generally acknowledged to be around 5 % - 10 % for APIs, depending on the crystal properties, such as size and shape [38,39,161–163]. The sensitivity of DSC to crystallinity can be better, with limit of detection around 2 % [39,162]. The sensitivity of both techniques is dependent on the sample, running time and refinements of the methodology, such as modulated or normal DSC. As the drug loading used here was only 10 % a considerable amount had to be crystallised in order to reach the LOD for either technique.

The limitations of using PLM as a main detection method compared to DSC and XRPD is that it is not quantitative and requires some interpretation. The result can therefore be dependent on the analyst. The decision, whether a bright mark on a sample is a crystal or an artefact of the sample can be subjective. However, here three approaches were used to minimise risk of misclassification of amorphous / crystalline material with PLM. Firstly, a clear protocol was established on how to distinguish between crystals and sample artefacts. Secondly, all measurements were performed by the same observer and thirdly measurements were repeated on all formulations at all time-points to confirm crystallinity from previous measurements.

2.6.2. Glass forming ability

All of the ten APIs investigated in this study belonged to class II or class III glass forming ability (GFA). Half were non-stable glass formers, class II (aprepitant, celecoxib, estradiol, nifedipine and orlistat) and the others were stable glass formers, class III (felodipine, fenofibrate, itraconazole, probucol and ritonavir) [43,46,56]. When considering only the glass forming ability of the APIs tested, a distinction between the two classes would be expected. Class III drug's stability would be expected to be less dependent on the selected polymer and manufacturing method. The stable glass formers would be expected to stay amorphous independent of formulation, as they make stable glasses on their own. In contrast class II substances should be more affected by factors of the formulation such as carrier and manufacturing method, being unstable glass formers. However, in reality no difference was observed between the two classes when the stability of each formulation is considered (Figure 22). When the average stability of each class is compared it is clear that there is no difference between the classes, as is shown in Figure 22.

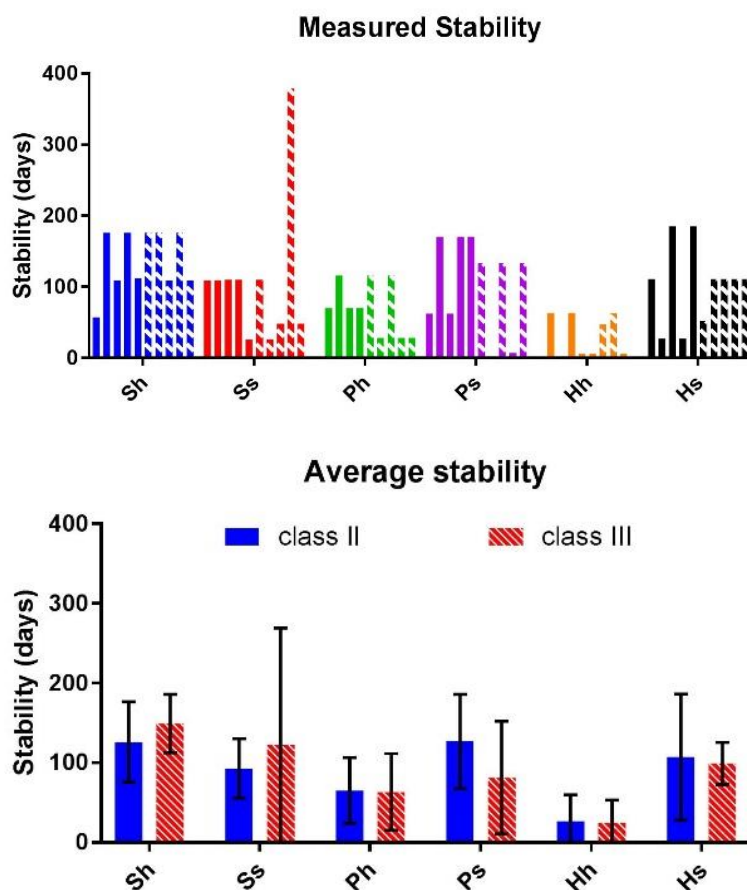


Figure 22 Top, measured stability of each formulation. Each column represents one formulation of one API, first half, in each colour, is class II and second half are class III APIs (striped). Bottom, shows same information but average stability of the five formulations in each class, error bars represent standard deviation.

GFA has been considered as a tool for formulation development. Depending on API's glass forming ability, amorphous formulations can be regarded a viable option [43,46,47,56,101]. However, most studies looking at GFA consider the API alone and not in a formulation. Here, no difference was observed between class II and class III in the range formulations produced. No APIs from class I were included as they were not expected to make stable solid dispersion formulations. However, following these results, it would be interesting to see whether a difference in stability would be observed between glass formers and non-glass formers in solid dispersion formulations.

2.6.3. Stabilising interactions

Generally, there are four different types of specific interactions that can occur between an API and a polymer, in order of decreasing bond strengths: ionic bonds > hydrogen bonds > dipole–dipole interactions > van der Waals interactions. The typical bond energy of these interactions and their relative strength are shown in Table 6. Ionic bonds are formed from strong attractions between two oppositely charged molecules. A hydrogen bond (H-bond) is an electrostatic interaction between a hydrogen and an electronegative atom (O, N or F), these can occur both between two identical molecules (API-API) and different molecules (polymer-API). A dipole-dipole interaction can occur between two polar molecules, when they line up so that the positive part of one molecule is opposite the negative one of the other molecule. The weakest of the interactions is the van der Waals interactions, which are induced dipolar interactions that can occur between any functional groups. Specific interactions between drug and polymer can impact the physical stability of solid dispersions. For instance, H-bonds between polymer and drug can hinder crystallisation by reducing the amount of drug in the conformation needed for crystallisation. H-bonding can also occur between APIs, which can facilitate crystallisation. However, if there are more or stronger H-bonds available between API and polymer, or amorphous API-API than between crystalline API-API this can impede crystallisation [41,153,164].

Table 6 Typical bond energy and relative strength of common intermolecular interactions in solid dispersions [165].

<i>Type of interaction</i>	<i>Bond Energy (kJ/mol)</i>	<i>Approximate relative strength</i>
<i>Ionic interaction</i>	850 – 1700	1000
<i>Hydrogen bonding</i>	50 – 170	100
<i>Dipole – dipole interaction</i>	2 – 8	10
<i>Van der Waals interaction</i>	~1	1

A previous study comparing the stability of two APIs, griseofulvin and indoprofin, in a solid dispersion with PVP, investigated the effect of H-bonding on stability. These APIs were chosen due to their lack of stability in the amorphous state and the inherent difference in chemistry. Mainly, griseofulvin has no HBD while indoprofin does. Fourier transform infrared spectrometry (FT-IR) was used to confirm the presence of H-bonding in indoprofin – PVP solid dispersions and the absence of these in dispersions containing griseofulvin. Indoprofin-PVP dispersions with 10% drug load were stable for the whole 90 days tested, when stored at 40 °C/69% RH. Griseofulvin, on the other hand, recrystallized within 10 days from the PVP dispersion when kept at the same conditions [132].

In another study of solid dispersions, these containing vitamin C and several polymers the influence of H-bonding between polymer and drug on physical stability was considered. As well as H-bonding, the effect of polymer hygroscopicity and T_g were examined. It was found that the formation of strong H-bonds between the polymer and drug in dispersions was the most important factor inhibiting crystallisation of vitamin C. The hygroscopicity of the polymer played some part in stability, especially when the solid dispersions were stored at a high RH. No correlation could be found between polymer T_g and physical stability of solid dispersions [116]. In a similar study performed by Van den Mooter *et al.* it was observed that in absence of any polymer-drug interaction, polymer T_g was an important stabilising factor [125].

In a recent study the effect of five polymers (guar gum, pectin, κ -carrageenan, gelatin, and PVP) on the crystallisation inhibition of thiamine chloride hydrochloride (THCl) in a solid dispersion was investigated. No direct link could be found between the hygroscopicity or T_g of polymers and stability of solid dispersion. However, polymers that showed H-bonding with THCl or possible ionic interactions were observed to be good at stabilising the amorphous form. In fact, PVP, the only polymer with no HBD resulted in the least stable dispersion [133].

Functional groups that can act as H-bond acceptors or donors are present in all three polymers studied here. H-bond donors include alcohol and carboxylic acid both present in HPMCAS. Amide (PVP-VA, SOL), esters (SOL, PVP-VA and HPMCAS) and ethers (HPMCAS, SOL) can all act as H-bond acceptors [158]. Thus only HPMCAS has H-bond donors while all polymers have H-bond acceptors present, see overview in Figure 14. The number of H-bond donors and acceptors are listed in Table 3 for each of the APIs. It can be observed that these numbers vary greatly between the different drugs, H-bond acceptors range from two to nine while H-bond donors range from zero to four. If the stability of solid dispersions was mostly dependent on the ability of the drug and polymer to make specific bonds such as H-bonds it would be expected that the drugs with large number of H-bond acceptors would be more stable in HPMCAS than PVP-VA and SOL while drugs with no H-bond donors would not be sufficiently stabilised by PVP-VA and SOL as no interaction could be made. Itraconazole, which has the greatest number of H-bond acceptors, first showed signs of crystallisation at 16 and 24 weeks, respectively for HME and spray-dried HPMCAS solid dispersions. However, both aprepitant and ritonavir were very unstable in HME HPMCAS and yet both have six H-bond acceptors. Fenofibrate, which has no H-bond donors, was the least stable spray-dried formulations for both SOL and PVP-VA but the HME SOL was stable through six months. It is therefore clear that there are further properties that contribute to the stability beyond just the ability of drug and polymer to interact, even though that is probably an important factor.

2.6.4. Effect of water on stability

Water, once absorbed into the formulation, can disrupt H-bonding between the drug and polymer. Furthermore water can act as a plasticizing agent, lowering overall T_g and thus increase molecular mobility [53,166–168]. Therefore, even if a solid dispersion formulation started out as stable with an abundance of stabilising H-bonds, at accelerated conditions these might be removed by water and thus cause instability and crystallisation.

In a recent study a new method was developed to detect phase separation in solid dispersions. Polymer- and API-specific fluorescent dyes were used in combination with a confocal fluorescent microscope to evaluate the homogeneity of solid dispersions. This method was used to investigate the influence of water uptake on phase separation. Solid dispersions containing PVP-VA and miconazole were subjected to high RH or immersed in a buffer solution. The sensitive method of the fluorescent microscope showed that water caused substantial phase separation of drug and polymer. This was likely to be due to the water acting as an antisolvent for the hydrophobic miconazole [126].

In most cases for SOL and PVP-VA, crystallisation was not observed while the spray-dried formulations were still a dispersible powder but only once they had transformed into a solid glass, which indicates the presence of water. PVP-VA is a very hydrophilic polymer and thus quite hygroscopic, absorbing 10% water at 50% RH. Relative to PVP-VA, HPMCAS is less hygroscopic, taking up 6-7% water at 75% RH [169]. The effect differences in hygroscopy have on the stability of the formulations can be seen clearly in the case of the spray-dried formulations above, especially when comparing formulations with PVP-VA and HPMCAS. PVP-VA spray-dried formulations form a glass after a while when exposed to very high humidity (75% RH). The powder seems to melt and/or dissolve into a glass and simultaneously crystals start forming. This transformation of powder into glass did not occur for the HPMCAS spray-dried formulations, even after one year at high humidity. It was deduced that this transformation was mainly due to the hygroscopy of the PVP-VA polymer, as it was not seen at less humid conditions. Despite the transformation of spray-dried formulations due to uptake of water for PVP-VA formulations they were still overall, more stable than corresponding HME formulations.

2.6.5. Manufacturing method

Some effects on stability could be due to the processing method used. In some cases, a reason might be quite clear, such as aprepitant being the least stable drug in HME formulations. Aprepitant has a very high melting point, 255 °C and was extruded at a lower temperature (160°C). Other effects of the processing method are not as clear. Only in four cases was the time of crystallisation onset the same for the two methods in two similar formulations, these being SOL-itraconazole, SOL-estradiol, PVP-VA -itraconazole and PVP-VA – felodipine. In all other cases the onset of crystallisation time differed between the two methods. The largest difference was observed for SOL-fenofibrate, where the spray-dried formulation showed signs of crystals after eight weeks while no crystals were observed in the HME until after one year.

When comparing the two processing methods it can be observed that overall, for SOL, HME seems to produce more stable solid dispersions than spray-dried, while the opposite appears to be the case for both PVP-VA, and HPMCAS. This indicates that the processing method has a profound effect on the solid dispersion formed and hence its stability. It is unlikely that specific bonds would only be present using one method or the other, therefore relative stability must be directly linked with attributes of the processing methods. These might include, for example, residual solvents that can destabilise the solid dispersion, in case of spray-dried formulations.

The influence of processing method on the stability of an itraconazole – Eudragit (acrylic polymers often used in solid dispersion formulations) solid dispersion has been studied [170]. Comparing solid dispersions produced with three different methods, spray-drying, film-casting, and hot melt extrusion, the maximum drug loading that could be stabilised by Eudragit was 27.5% for spray-drying and 15% for each of the other two methods. This shows that the processing method can have a significant influence on the stabilisation of solid dispersions [170].

A comparison of HME and spray-dried solid dispersions, in regards to interactions, drug distribution and homology of the dispersions was made by Tian *et al.* [91]. Solid dispersions were made with felodipine and three polymers, SOL, HPMCAS and PVP using both HME and spray-drying. It was found that a wider range of drug loading, up to 80 %, was achievable using spray-drying compared to HME (up to 50 %). However, these dispersions produced using a spray-drying process were more likely to have API rich domains and recrystallize on heating in modular DSC (MDSC). This was assumed to be due to residual solvents in the formulation causing phase separation. HME was found to be more stable, no sign of recrystallization in MDSC up to 50 % drug loading. Furthermore, the HME formulations showed stronger interactions between polymer and API and a more homogenous distribution of the API in the dispersion. These factors would likely promote stability of the solid dispersion [91]. A similar study comparing the properties of spray-dried and HME miconazole – PVP-VA solid dispersions found comparable results. HME dispersions were better mixed and more homogenous than the spray-dried ones [171].

2.6.6. Stabilising Polymer

For some drugs there is a clear difference in stability between polymers while for others there is minimal difference. For instance, probucol was very stable in both formulations containing SOL. No crystals were observed up to six months in HME and it was still amorphous after one year in spray-dried formulations. Probucol was the only drug that stayed amorphous in spray-dried SOL for more than four months and the only formulation to stay amorphous for the whole year tested. However, for PVP-VA it was one of the first APIs to show signs of crystallisation in both spray-dried and HME formulations after 4 and 8 weeks storage respectively. In HPMCAS the crystallisation onset was determined to be 4 weeks for HME and 24 weeks for spray-dried. When considering the physicochemical properties of probucol, ClogP stands out as extreme. Probucol has a ClogP of 10, which is the highest of the APIs used in this study. It is possible that this attribute is connected with this difference in stability, the PEG and PCL parts of SOL may better stabilise this highly lipophilic molecule compared to the more hydrophilic PVP-VA, and the

amphiphilic HPMCAS [153]. Other drugs such as fenofibrate started crystallising at the same time for all three polymers, at 8 weeks for Hh and Ph and Ss.

When comparing the stability of spray-dried solid dispersions it is interesting to note that for each of the polymers, two APIs had shown signs of crystallisation by two months. However out of the six crystallised formulations only two contained the same drug. For HPMCAS it was celecoxib and nifedipine; probucol and fenofibrate for PVP-VA; and orlistat and fenofibrate for SOL. It can therefore be proposed that this difference is due to the stabilising effect of the polymer or a lack thereof. Even the least stable drugs behave quite differently depending on the polymer. This would indicate that relative stability is not solely due to the method used or the inherent instability of the API, but how well the polymer can influence the physical stability of the dispersed drug.

Using a polymer with a high T_g will increase the T_g of its solid dispersions as the API T_g values are generally quite low (here ranging from -45°C to 85°C). Solid dispersions with high T_g values are generally thought to be more stable than ones with a lower T_g [41]. Although some studies have found a strong correlation between increasing polymer T_g and decreasing crystallisation tendency, others have found no such connection or even shown an opposite trend [116,125,172,173]. In this study the three polymers used have a wide range of physicochemical properties, such as T_g , SOL 70°C , PVP-VA 103°C and HPMCAS 122°C [149,152,154]. The results show that generally SOL is the most stabilising polymer in HME formulations despite having the lowest glass transition temperature. The same does not apply to the spray-dried formulations where PVP-VA and HPMCAS show better crystallisation inhibition properties. The opposite has been noted in the literature, HME - high drug loaded felodipine - HPMCAS dispersion was found to be more stable than spray-dried [127]. However, PVP-VA and HPMCAS were spray-dried at a temperature below their T_g while for SOL this was above its T_g . Whether this affected stability is unclear. It is clear, however, that there are more factors that contribute to stability than just the polymer T_g .

When the mean stability of each combination of polymer and manufacturing method, that is Sh, SS, Ph, Ps, Hh and Hs, was compared, there was only a marked difference between Soluplus HME and HPMCAS HME, see Figure 23. Sh had the highest mean and overall a relatively low standard deviation (SD). Hh had the lowest mean stability and SD. As has been discussed above, each API will be stabilised differently by different polymer – manufacturing combinations and there are many factors that affect the stability of the formulations. This overview of mean stability might indicate that overall, Soluplus HME was best at stabilising APIs in solid dispersions while HPMCAS HME was the worst. However, this is only based on the result of ten APIs with large internal variance in results. Therefore, such conclusions should be taken more as suggestions than an absolute.

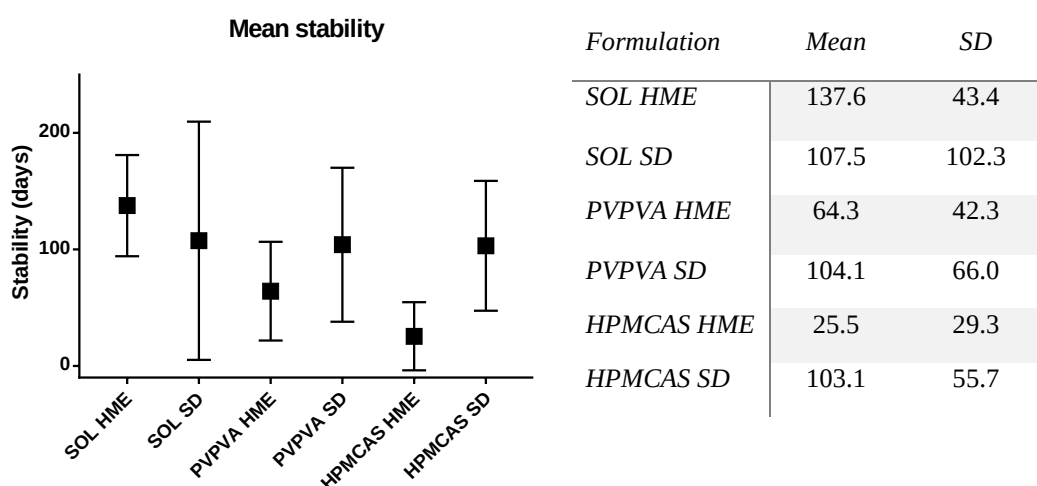


Figure 23 shows the mean stability of each polymer - manufacturing combination. The error bars represent the calculated standard deviation (SD).

2.7. Conclusions

60 solid dispersions were produced successfully using ten APIs, three polymers and two processing methods. An accelerated stability study has shown significant difference in time to crystallisation dependent on both choice of polymers and processing method. This extensive experimental dataset provides new insights into the complexity of the stability of solid dispersion systems.

There are many factors known to influence the stability of solid dispersions, as described above. However, from this large dataset it can be concluded that rather than a single factor being predominantly influential the stability is dependent on multiple factors working together or against each other. For instance, specific interactions between polymer and drug can stabilise the solid dispersion. Yet, the potential for H-bonding, as an example, does not guarantee increased stability, on the contrary, H-bonding between API molecules can facilitate crystallisation. In the absence of any specific interactions other factors such as T_g , physicochemical properties of the API and the manufacturing method may be more important.

However, there are some new conclusions that can be drawn, such as HPMCAS and PVP-VA are more likely to give a stable formulation using spray-drying, while HME seems to be more favourable for SOL solid dispersion formulations. This has not been noted on a large scale dataset before. Furthermore, rather unexpectedly, no difference in stability can be seen between formulations containing stable or non-stable glass formers.

Chapter 3 – Multiple linear regression models

3.1. Introduction

Choosing the most appropriate formulation route for a new drug is largely still based on formulator know-how and experimental screening methods. Properties of the API are often not considered, but rather in-house knowledge and availability of equipment steer the process [1,5]. Here it is proposed that a knowledge-based computational tool that could predict the best formulation strategy would greatly improve this process. Indeed, there are some support tools currently available that can be used to facilitate this process such as guidance maps, high through-put screening, and statistical models, as listed in Chapter 1. Using a support tool in the formulation development can shorten the development process, limiting the number of experiments required by indicating which formulation route is appropriate for a specific API and there remains a significant need in this area.

As the choice of polymer carrier and processing method are vital to the consequent stability of a solid dispersion, the use of a knowledge-based support tool in formulation development would be beneficial. Currently, although some effort has been put into building such tools, their availability remains quite limited [5,17]. Several models have been built to predict the glass-forming ability of APIs and some predict the stability of the amorphous form of APIs. These are intended to give an indication of whether a dosage form containing the API in amorphous form is a viable formulation choice [5,46,56,100,101,174]. Statistical models have also been built for use in formulation development, for choosing formulation route, predicting drug solubility in lipid excipients and miscibility in polymer for solid dispersions [18,37,57]. Recently molecular dynamics (MD) simulations have been used to predict T_g , to investigate interactions between drug and polymer in solid dispersions and the effect of water in these systems [96]. Historically the Flory-Huggins equation or Hansen solubility parameters have been used theoretically to analyse interactions and miscibility and provide an indication of likely stability.

The advancement of a computational tool that can compare several solid dispersion formulations and predict their stability has yet to be reported. Here a multiple linear regression (MLR) model was built to fulfil this need of a knowledge based support tools in the development of solid dispersion formulations.

Some effort has been put into creating models that can predict crystallisation properties of substances. Several machine-learning algorithms have been used for this purpose. Machine learning algorithms can be divided into two classes, supervised and unsupervised. Supervised learning is used to deduce a function from a dataset of input and output variables. It is built on training data and validated with test data, a new dataset previously unseen by the model. If the function outcome value is continuous, then the function deducing process is called ‘regression’. Alternatively, if the output variables is qualitative the process is called ‘classification’ [175]. An example of regression is a model to predict stability, while a model to predict whether an API can be crystallised would be a classification model, *e.g.* returning an outcome of amorphous/crystallised. Machine learning algorithms commonly used in supervised learning are based on linear regression, random forest and support vector machine in addition to multiple linear regression (MLR). Unsupervised learning does not consider output data but is used to find underlying patterns or distributions in the data. These approaches can be further divided into two groups, clustering and association. Clustering attempts to find inherent groups in the dataset while association finds rules that describe large portions of the data. Apriori and k-means are commonly used algorithms in unsupervised learning [175,176].

The resulting model will depend on which machine learning algorithm is used [5,177,178]. Some studies have built and compared several types of models from the same dataset and found one type superior to the rest. The superior algorithm depends, however, on the application and dataset, which is why all these methods are widely used. A study building models to predict glass transition temperatures of APIs found support vector regression gave the best result compared to artificial neural networks (ANN), partial least squares (PLS), principal component analysis

(PCA) and more [174]. When predicting solubility of an API one study found random forest superior to support vector machines (SVM), ANN and PLS [103] while a similar study found MLR the best choice from the four types tested [179].

MLR focuses on finding linear relationships between an outcome variable and more than one independent property. Regression analysis is one of the most frequently applied statistical methods. It has been applied in a range of scientific fields, including medicine, biology, economics, engineering and more. It has, for instance, been used to predict glass forming ability and amorphous stability [100]. MLR is transparent, especially compared to some other algorithms, as it shows how, and to what extent, each covariate affects the outcome. The three-fold purpose of regression models are to: find correlations between parameters (x) and response variable (y), predict response (y) based on parameters (x) and through screening of variables (x) find which parameters best explain response variable (y) to better understand the relationship. MLR is not possible when the number of variables is higher than the number of observations, such as is common in the pharmaceutical area. In these cases variable selection must precede the MLR process [175,180].

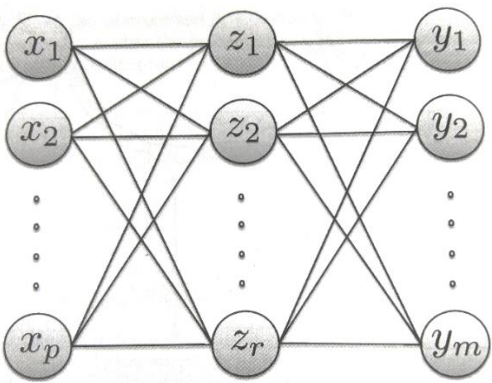
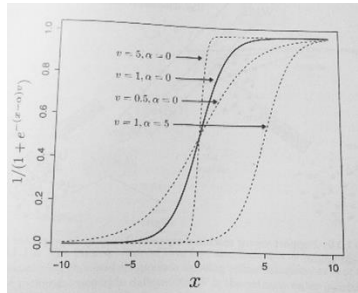
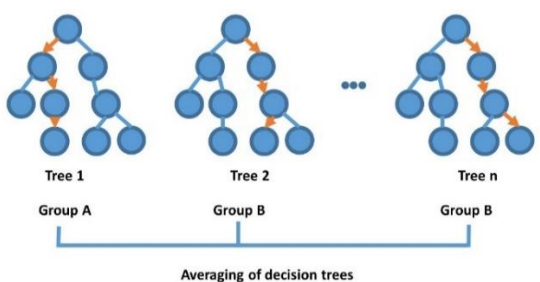
Random forest (RF) uses a collection of decision tree predictors. The idea is to make a large number of random decision trees and then use the average of these as the RF model. Through its design it has inbuilt LOOCV. RF has been used in various pharmaceutical and life science applications such as to predict crystallisation tendency of both APIs and proteins [176], and conducting QSAR studies [105,181].

Support vector machines (SVM) are related to artificial neural networks. They can be used on linear or non-linear data and for both classification and regression analysis. SVM constructs one or more hyperplanes that separate different groups. Ideally, the separation between groups should be complete so observations from the two groups do not overlap. However, this may not always be possible [46,174,176].

Partial least squares (PLS) is commonly used in pharmaceutical applications. This is due to there often being a large number of independent covariates that are highly correlated, such as when considering molecular descriptors of APIs. PLS assumes a few variables account for most of the variance in the dataset. Both the input and output variables are mean centred and scaled to unity of variance, before creating a number of linear combinations. From these the covariates that have the highest variance and the most effect on, the output variables are chosen. Thus the PLS seeks to explain the data variance while taking into account the covariants effect on the response variable. PLS models have been developed to predict glass forming ability (GFA) [101], solubility advantage by amorphisation [182], API solubility in FaSSIF [104], API solubility in lipid excipients [37] and formulation route selection [18]

Neural networks or artificial neural networks (ANN) is a non-linear statistical model that can be used for both regression and classification. It simulates the structure of biological neural networks like the brain. Artificial neurons are nodes with weights as directed connection between the output of one neuron to the input of the next. ANN is used to find correlations and patterns that are otherwise hard to identify. However, ANN can have hidden layers of networks which make them less transparent than other types of models [175]. ANN has been used to predict dissolution profiles for tablets [183] and drug release from solid dispersions [102].

Table 7 Overview of supervised machine learning algorithms commonly used, figures from [175].

<u>MLR</u> - Assumes linearity of parameter function - Assumes independence of observations - Require non-linear independence of covariates - Separate parameter selection - Transparency	<u>PLS</u> - Assumes linearity of parameter function - Assumes independence of observations - Scaling of input and output data - Inbuilt parameter selection - Strong inter variable correlations allowed
<u>ANN</u>  - Can handle non-linear datasets - Non transparent	<u>SVM</u>  - Related to ANN - Non transparent
<u>RF</u>  In built LOOCV	<u>Unsupervised machine learning</u> - Finds patterns and correlation within a dataset unrelated with an outcome. - Cannot predict output - Commonly used algorithms: - PCA and clustering

While all the types of machine learnings described above are commonly used in pharmaceutical modelling, MLR was the method chosen for this study. MLR is the simplest form of modelling, it is very transparent – shows the effect of each covariate on the output variable, and can be readily accessed through the freeware R.

3.2. Materials and methods

3.2.1. Physicochemical properties of APIs

The program Instant JChem from ChemAxon was used to calculate and predict physicochemical properties of the APIs. These were based on the molecular structure of each API. Furthermore information on the melting point (mp) of each drug was obtained from Chemspider [184], the T_g in ° C of each formulation was measured with DSC and predicted using the Fox equation (see Equation 3) .

$$\frac{1}{T_g} = \frac{w_1}{T_{g1}} + \frac{1 - w_1}{T_{g2}}$$

Equation 3 The Fox equation is very similar to the Gordon - Taylor equation and uses the T_g and weight fraction (w) of each component, API and polymer to calculate the T_g of the solid dispersion.

The Fox equation was used rather than the Gordon – Taylor equation (Equation 4) which is also commonly used, due to unknown density values of some substances. The K constant is calculated from the density (ρ) and T_g of each component. ($K = T_{g1} \rho_1 / T_{g2} \rho_2$).

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

Equation 4 Gordon - Taylor equation uses the T_g of polymer and API along with the weight fraction (w) of each and an adjustable fitting parameter K to predict a T_g of a solid dispersion.

The following parameters were included in the modelling process: molecular weight (MW), lipophilicity (ClogP) determined using the ChemAxon method, strongest basic pK_a (b.pKa), topological polar surface area (tPSA), number of atoms (nAtom), number of bonds (nBond), number of chiral atoms (nChir), number of H-bond acceptors (HBA), number of H-bond donors (HBD), number of rings (nRing), number of nonterminal rotatable bonds (nRot), number of aliphatic atoms (nAliph), number of aromatic atoms (nAr), water accessible surface area (ASA), hydrophilicity-lipophilicity balance (HLB), intrinsic water solubility in $\log_{10}$ of molar solubility ($\log S_0$), molecular polarizability (molPol), van der Waals surface

area (vdW-SA), melting point (mp), number of aliphatic rings (nAliphRing), number of aromatic rings (nArRing), number of carbon atoms (nC), number of fluorine atoms (nF), number of nitrogen atoms (nN), number of oxygen atoms (nO), number of sulphur atoms (nS), number of chlorine atoms (nCl), number of heteroatoms (atoms other than carbon and hydrogen; nHet), number of heteroatoms divided by number of carbons (nHet/C), number of sp^3 carbon atoms, number of sp^2 carbon atoms. The strongest acidic pKa value was not used in the modelling, as not all APIs had values for these and the modelling process did not allow missing values. The physicochemical properties can broadly be divided into four groups depending on what characteristics of the API they describe, see Table 8. These are size and shape of the API, flexibility or complexity of the molecule, hydrogen bonding and finally the API's lipophilicity, solubility and other characteristics.

Table 8 the physicochemical properties of the APIs can be divided into four groups based on what characteristics of the API they describe. The four groups are Size and shape, flexibility and complexity, hydrogen bonding and lastly lipophilicity, solubility and other

Size / shape	Flexibility / complexity	Hydrogen bonding	Lipophilicity, solubility and other
nAliph, nAr, ASA, nAtom, nBond, nHet, MW, molPol, nC, nCl, nS tPSA, vdW-SA, nHet/C	nAliphRing, nArRing, nChir, nRing, nRot, sp2, sp3,	HBA, HBD, nF, nO, nN,	HLB, logS0, logP, mp, bpKa

Furthermore two properties of the solid dispersions were included, measured T_g ($T_{g,meas}$) and predicted T_g ($T_{g,pred}$) in °C, where the Fox equation was used to calculate the expected T_g of the solid dispersion. The predicted value was also used as a $T_{g,meas}$, in those instances where a T_g could not be measured. The physicochemical properties used and their values are listed in Table 9 and the measured and predicted T_g are listed in Table 10.

Table 9 Calculated and predicted parameters used in the building of the MLR models, ^a from [174]

Physicochemical property	Nifedipine	Felodipine	Itraconazole	Probucol	Ritonavir	Fenofibrate	Celecoxib	Estradiol	Orlistat	Aprepitant
<i>nAliph</i>	19	19	21	23	28	13	9	14	35	20
<i>nAliphRing</i>	1	1	2	0	0	0	0	3	1	1
<i>nAr</i>	6	6	28	12	22	12	17	6	0	17
<i>nArRing</i>	1	1	5	2	4	2	3	1	0	3
<i>ASA (Å²)</i>	533	583	976	874	981	642	602	392	985	679
<i>nAtom</i>	43	44	87	83	98	46	40	44	88	58
<i>avPol (Å³)</i>	33	37	74	62	76	38	35	32	61	44
<i>nBond</i>	44	45	93	84	101	47	42	47	88	61
<i>nChir</i>	0	1	3	0	4	0	0	5	4	3
<i>HBA</i>	5	3	9	2	6	3	3	2	3	6
<i>HBD</i>	1	1	0	2	4	0	1	2	1	2
<i>nHet</i>	8	7	14	4	13	5	9	2	6	14
<i>nHet/C</i>	0.47	0.39	0.40	0.13	0.35	0.25	0.53	0.11	0.21	0.61
<i>HLB</i>	16	11	5	2	4	4	4	2	3	16
<i>logS₀ (M)</i>	-3	-4	-9	-11	-8	-6	-6	-4	-11	-6
<i>logP</i>	1.82	3.44	7.31	10.57	5.22	5.28	4.01	3.75	8.11	4.52
<i>MW</i>	346.3	384.3	705.6	519.8	721.0	360.8	381.4	272.4	495.8	534.4
<i>molPol (Å³)</i>	33	37	71	61	75	38	36	31	56	43
<i>mp (°C)</i>	173	145	166	125	121	81	158	152	44	255
<i>nC</i>	17	18	35	31	37	20	17	18	29	23
<i>nCl</i>	0	2	2	0	0	1	0	0	0	0
<i>nF</i>	0	0	0	0	0	0	3	0	0	7
<i>nN</i>	2	1	8	0	6	0	3	0	1	4
<i>nO</i>	6	4	4	2	5	4	2	2	5	3
<i>nS</i>	0	0	0	2	2	0	1	0	0	0
<i>nRing</i>	2	2	7	2	4	2	3	4	1	4
<i>nRot</i>	6	6	11	8	18	7	4	0	23	8
<i>sp²</i>	12	12	22	12	21	14	15	6	3	14
<i>sp³</i>	5	6	13	19	16	6	2	12	26	9
<i>b.pK_a</i>	-6.63	-6.64	3.92	-5.08	2.84	-4.93	0.08	-0.88	-1.44	3.74
<i>tPSA (Å²)</i>	107.8	64.6	100.8	40.5	145.8	52.6	78.0	40.5	81.7	75.2
<i>vdW-SA (Å²)</i>	466	493	965	916	1,065	533	484	437	925	656

Table 10 Measured and predicted T_g (°C) values for each formulation. The measured values are an average of three measurements. The predicted values ^p, below the dashed line, are calculated using the Fox equation. NA - where no Tg was observed.

	<i>Nifedipine</i>	<i>Felodipine</i>	<i>Itraconazole</i>	<i>Probucol</i>	<i>Ritonavir</i>	<i>Fenofibrate</i>	<i>Celecoxib</i>	<i>Estradiol</i>	<i>Orlistat</i>	<i>Aprepitant</i>
<i>Sh</i>	70.2	68.2	68.5	68.9	66.6	56.1	72.7	64.2	NA	74.3
<i>Ss</i>	71.2	67.8	70.0	67.0	68.3	57.5	71.7	68.0	NA	74.2
<i>Kh</i>	102.3	100.9	103.6	101.4	96.3	84.8	104.8	103.1	NA	106.0
<i>Ks</i>	104.8	104.5	109.9	103.4	102.5	91.8	103.7	99.5	NA	110.6
<i>Hh</i>	105..3	103.4	109.0	101.7	97.6	92.7	102.0	104.7	NA	NA
<i>Hs</i>	103.7	103.7	109.3	104.3	102.6	91.1	103.7	100.0	94.8	110.0
<i>Sh^p</i>	67.5	67.5	68.8	65.7	67.9	61.1	68.8	71.5	58.5	66.6
<i>Ss^p</i>	67.5	67.5	68.8	65.7	67.9	61.1	68.8	71.5	58.5	66.6
<i>Kh^p</i>	95.4	95.4	96.7	93.6	95.8	89.0	96.7	99.4	86.4	94.5
<i>Ks^p</i>	95.4	95.4	96.7	93.6	95.8	89.0	96.7	99.4	86.4	94.5
<i>Hh^p</i>	112.5	112.5	113.8	110.7	112.9	106.1	113.8	116.5	103.5	111.6
<i>Hs^p</i>	112.5	112.5	113.8	110.7	112.9	106.1	113.8	116.5	103.5	111.6

3.2.2. Multiple linear regression models

The publicly available software, R [185] and R studio (version 1.0.143) were used to build the multiple linear regression models. The calculated and predicted parameters (see Table 9) were used without units as input variables in to the model equations. The measured stability (time to crystallisation), the output, was used as the $\log_{10}$ value, stability in days + 1 in order to avoid zero values. Using the leaps package in R an exhaustive selection of covariates was made with one, two and three as a maximum number of covariates; using backwards, forwards, stepwise, and sequential replacement. This insured that the simplest, most effective model could be made, so that each parameter improves the model more than can be expected by chance. To avoid linear dependency of covariates, the selected parameters were tested for intercorrelation and further iterations of parameter selection were made if this was found to be higher than 0.9. Once three uncorrelated parameters had been selected, these were used to build a model that linked the physicochemical properties with the logarithm of the measured stability, *i.e.* time until onset of crystallisation. An overview of the modelling process is presented in Figure 24.

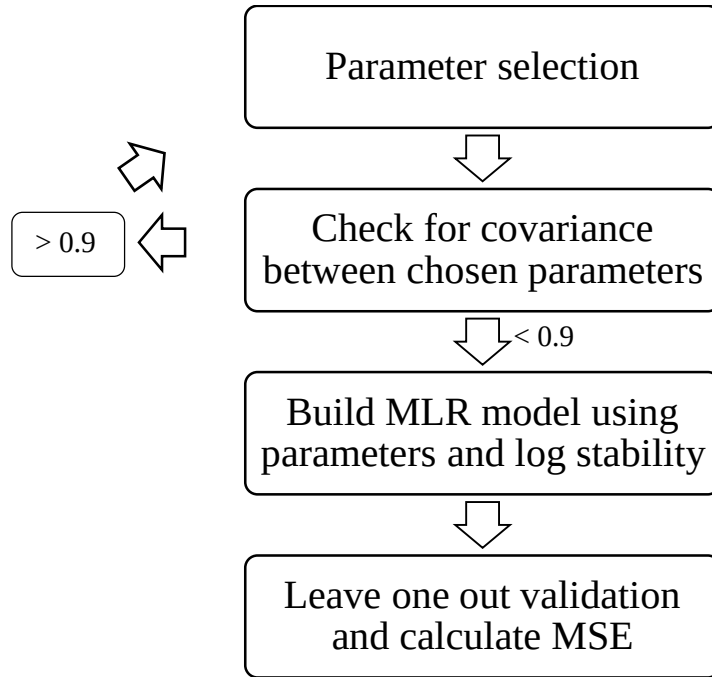


Figure 24 Overview of the modelling process

3.2.2.1. Validation

As this was a relatively limited dataset (in terms of statistical analysis) of ten observations per model, the whole dataset was used to build the models, rather than a training and test set method. A leave-one-out cross validation (LOOCV) was performed on each model to test its predictability and to calculate the mean square error (MSE). The whole process was repeated for each dataset (combination of polymer and processing method), resulting in six MLR models, for each number of covariates. An outlier calculation was performed on the squared error from LOOCV. Points outside of the limits calculated were classed as outliers and left out of the MSE calculations. The limits were calculated from the 1st and 3rd quartile and the difference between those, (inter quartile range, IQR) according to Equation 5 [186].

$$\text{Lower} = 1\text{st Quart} - 1,5 \times \text{IQR}$$

$$\text{Upper} = 3\text{rd Quart} + 1,5 \times \text{IQR}$$

Equation 5 the upper and lower limits for outlier detection were calculated from the 1st and 3rd quartile and the inter quartile range.

3.2.2.2. External validation

An external validation will better assess the true predictability of the model and give indications of overfitting, compared to a LOOCV. An experimental, external dataset was obtained from the literature. The following criteria must be met for an external dataset. 1) Use one or more of the polymers used here, SOL, PVP-VA or HPMCAS, 2) contain a new API, with a known molecular structure, not included in the dataset the model was built on, 3) have an element of comparison, that is either one drug in multiple polymers or in both manufacturing methods.

A detailed search of the literature found one study that fulfilled all criteria [187].

3.3. Results

3.3.1. Models

Six statistical MLR models were constructed, one for each combination of polymer and manufacturing method. Even though each modelling process started with the same predicted, calculated, or measured parameters, none of the models were identical, each indicating that different parameters were influential in the stability of that particular polymer dispersion and manufacturing method. This provides a direct indication of why it has been difficult to find agreement among different studies on the most important parameters to consider when seeking the most stable solid dispersion formulation and production method.

Due to the relatively limited dataset (although very large compared to previous studies), the number of independent physicochemical parameters in each model was limited to three. Models containing one and two parameters were also built as well as models containing pooled datasets. The first step was to determine which three parameters had the strongest correlation with the stability of the formulations, see Figure 25.

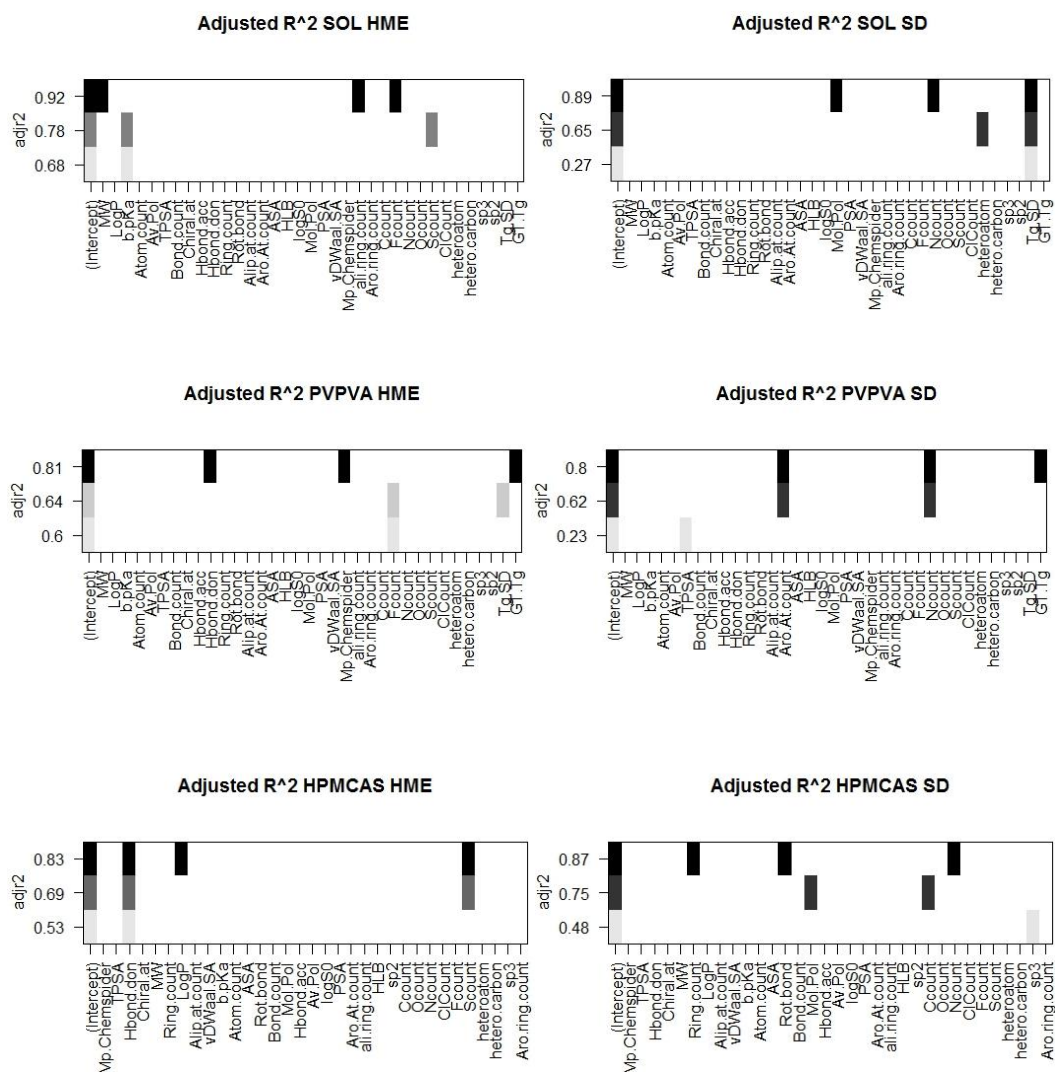


Figure 25. The most influential parameters indicated in each graph, plotted against the R^2_{adj} . Light grey shows the single most descriptive parameter, dark grey the two best parameters and black indicates which three parameters in combination best explain the difference in stability for each dataset. As expected the R^2_{adj} increases with the addition of parameters in all datasets.

Once the three most influential parameters had been chosen, a MLR model was derived using the stability of the ten drugs and the parameters. The models, adjusted R^2 (R^2_{adj}) and mean square error are listed in Table 11. The Sh model stability shows a negative correlation with the number of H-bond acceptors and the number of chiral atoms, and a positive correlation with topological polar surface area. If the number of F atoms was included in the model the adjusted R^2 was 0.918 and the MSE was 0.08, the model was then $\log(\text{stability}) = 2.675 - 0.04 \text{ nF} - 0.132 \text{ nAliphRing} - 0.001 \text{ MW}$. Ss formulation stability is positively correlated with

measured glass transition temperature ($T_{g,meas}$) and molecular polarizability while negatively correlated with the number of N atoms in the drug. Stability of Ph shows a positive correlation with predicted glass transition temperature ($T_{g,pred}$) and a negative correlation with number of H-bond donors and the melting point of the API. The nitrogen count and $T_{g,pred}$ are positively correlated with stability of Ps while the number of aromatic atoms shows a negative correlation. The Hh stability shows negative correlation with number of HBD and lipophilicity whilst the sulphur count is positively correlated. Both ring count and number of rotatable bonds is positively correlated with the stability of HPMCAS spray-dried but the number of nitrogen atoms is negatively correlated. The optimal models have R^2_{adj} values between 0.798 and 0.895.

Models were also made using only one and two parameters (Table 11), these had lower R^2_{adj} (0.231-0.659) and (0.621-0.781) respectively but in most cases lower MSE (0.029-7.445) and (0.068-2.619). Attempts to build models using pooled data were unsuccessful, with R^2_{adj} ranging from -0.1 to 0.33. These included six pooled datasets, one including all data, one for each method – containing data from all polymers and one for each polymer which included both methods.

Table 11. Summary of models, including adjusted R2 and MSE values. The following abbreviations are used, nCov – number of covariants, R2adj – adjusted R2, MSE – mean square error, logStab – log Stability, tPSA – topological polar surface area atoms, HBA – H, mp – melting point, nF – number of F atoms, nS – number of S atoms, nN – number of N atoms, HBD – number of H bond donors, nRot – number of rotatable bonds, molPol – molecular polarizability, Tgpred – predicted Tg using the Fox equation, Tgmeas – measured Tg, nRing – number of rings, nAr – number of aromatic atoms, nHet – number of heteroatoms, sp3 – number of sp3 carbon atoms.

	nCov	Model	R ² _{adj}	MSE
Sh	3	$\logStab = 2.307 + 0.003 \text{ tPSA} - 0.055 \text{ nChir} - 0.082 \text{ HBA}$	0.891	0.08
Ss	3	$\logStab = -3.864 + 0.081 \text{ T}_{g,\text{meas}} - 0.163 \text{ nN} + 0.015 \text{ molPol}$	0.895	0.27
Ph	3	$\logStab = -11.2106 - 0.2355 \text{ HBD} - 0.0112 \text{ mp} + 0.1536 \text{ T}_{g,\text{pred}}$	0.815	1.02
Ps	3	$\logStab = -5.252 + 0.079 \text{ T}_{g,\text{pred}} + 0.390 \text{ nN} - 0.119 \text{ nAr}$	0.798	2.11
Hh	3	$\logStab = 2.472 - 0.849 \text{ HBD} - 0.124 \text{ ClogP} + 0.771 \text{ nS}$	0.827	3.48
Hs	3	$\logStab = 0.953 + 0.055 \text{ nRot} + 0.331 \text{ nRing} - 0.216 \text{ nN}$	0.870	0.78
Sh	2	$\logStab = 1.988 + 0.078 \text{ nS} - 0.042 \text{ pKa}_b$	0.781	0.07
Ss	2	$\logStab = -1.829 - 0.059 \text{ nHet} + 0.063 \text{ T}_{g,\text{meas}}$	0.647	0.31
Ph	2	$\logStab = -0.605 - 0.232 \text{ nF} + 0.025 \text{ T}_{g,\text{meas}}$	0.651	1.52
Ps	2	$\logStab = 2.116 - 0.104 \text{ nAr} - 0.389 \text{ nN}$	0.621	1.05
Hh	2	$\logStab = 1.754 - 0.711 \text{ HBD} + 0.480 \text{ nS}$	0.688	2.62
Hs	2	$\logStab = 1.415 + 0.060 \log P + 0.102 \text{ nChir}$	0.700	0.28
Sh	1	$\logStab = 2.033 - 0.042 \text{ pKa}_b$	0.659	0.03
Ss	1	$\logStab = -0.667 + 0.038 \text{ T}_{g,\text{meas}}$	0.272	0.39
Ph	1	$\logStab = 1.831 - 0.199 \text{ nF}$	0.605	0.39
Ps	1	$\logStab = 0.839 + 0.012 \text{ tPSA}$	0.231	7.45
Hh	1	$\logStab = 1.669 - 0.479 \text{ HBD}$	0.534	0.52
Hs	1	$\logStab = 1.599 + 0.030 \text{ sp}^3$	0.483	0.15

The MSE was calculated from a leave-one-out validation of each model. In a leave-one-out cross validation (LOOCV) one of the data points (formulations) is excluded from the dataset used to build the model. The same procedure was used as with the whole dataset and the parameters selected before the model is built. For example,

a model was built leaving out the nifedipine data. The new model was then used to predict the stability of the drug that was excluded, nifedipine. This was repeated ten times, each time excluding one API and predicting the stability of that API. The MSE of these predictions was then calculated and it gives an indication of the predictability of the models. The comparison of predicted and measured stability from each LOOCV can be seen in Figure 27 for three covariate models. Substantial difference in MSE can be seen between the models (see both Figure 27 and Table 11), the lowest MSE is in the Sh model, 0.08, while the highest is seen in the Hh model, 3.48.

During the LOOCV process a covariate selection is done for each new model. The chosen covariate can differ from the model built on the whole dataset. Each of the models selected in the LOOCV process for SS, is shown in Table 12 along with the overall model, the squared error between the predicted and measured value and the calculated MSE.

In the LOOCV some errors calculated were extremely large, this was especially the case where the API left out had an extreme value in the covariates chosen for the model. This can be seen for orlistat in SS, Table 12, where its stability is poorly predicted due to its extremely low T_g . These values greatly obscured the MSE. The errors were therefore checked for outliers as shown in Table 13. The values in bold were found to be outliers and at the bottom of the table the corrected MSE is stated where the outlier values have been left out. This can also be seen depicted in Figure 26.

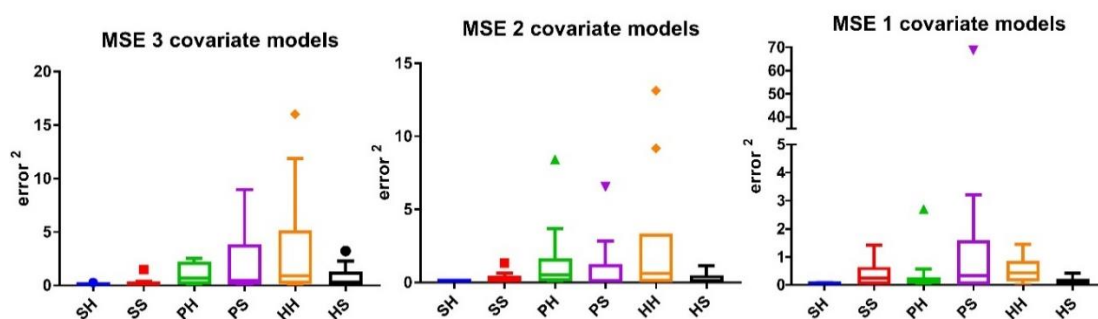


Figure 26 The average squared error for each model, with one, two and three covariates, calculated from LOOCV. Points outside whisker represent outliers. Thus no outliers are found PH and PS three covariate models but in both one and two covariate models show outliers.

Table 12 LOOCV models, R^2_{adj} and error for SS. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. The orlistat model gives much larger error than the rest. Orlistat is poorly predicted as it has the lowest T_g and fewer number of aromatic rings than the rest of the APIs or none compared to 1-5. Abbreviations used: int – intercept, k – constant of covariate.

	<i>int</i>	<i>k</i>	<i>Mol.Pol</i>	<i>k</i>	<i>nN</i>	<i>k</i>	$T_{g,meas}$	R^2_{adj}	<i>Meas</i>	<i>Pred</i>	<i>Error</i> ²
SS	-3.8637	0.01512		-0.163		0.08081		0.89			
<i>Nifedipine</i>	-0.3768	-0.0082	TPSA	0.23089	nS	0.04112	71.2	0.90	2.05	1.67	0.14
<i>Felodipine</i>	-3.3164	0.07286	ClogP	-0.099	1	0.07509	67.8	0.90	2.05	1.93	0.01
<i>Itraconazole</i>	-4.1345	0.0155	71	-0.1928	8	0.08522	70.0	0.94	1.69	1.39	0.09
<i>Probucol</i>	-1.8606	-0.0164	nF	-0.0604	0	0.05745	67.0	0.97	2.58	1.99	0.35
<i>Ritonavir</i>	-3.9781	0.01715	75	-0.1607	6	0.08127	68.3		1.69	1.89	0.04
<i>Fenofibrate</i>	2.01778	-0.1377	nRing	-0.0823	nRot	0.00162	vdW-SA	0.90	1.43	1.17	0.35
<i>Celecoxib</i>	-3.8615	0.01577	36	-0.1655	3	0.08029	71.7	0.89	2.04	1.96	0.01
<i>Estradiol</i>	-3.7694	-1.8758	nHet/C	-0.084	0	0.09779	68.0	0.97	2.05	2.67	0.39
<i>Orlistat</i>	-1.918	-0.124	logS0	-0.2705	nArRing	0.05483	58.5	0.97	1.43	2.65	1.49
<i>Aprepitant</i>	-3.7032	0.07092	ClogP	-0.1028	4	0.08163	74.2	0.94	2.04	2.27	0.05
										MSE	0.29

Table 13 Calculations of outliers. Table shows all errors calculated from LOOCV of each three covariate model. From the 1st and 3rd quartile the inter quartile range was calculated (IQR). The upper and lower values represent the limits for outliers (Lower = 1Q - 1.5*IQR and Higher = 3Q + 1.5 * IQR) [186]. The error values in bold are outliers according to these calculations. At the bottom are the mean and corrected mean where the outliers have been removed.

	<i>SH</i>	<i>SS</i>	<i>KH</i>	<i>KS</i>	<i>HH</i>	<i>HS</i>
1	0.11	0.14	0.13	0.1	0.43	0.14
2	0	0.01	0.06	0.01	0.18	0
3	0.09	0.09	0.77	0.43	11.87	0.12
4	0.27	0.35	0.01	0.02	1.2	0.11
5	0.03	0.04	2.53	1.32	16.02	2.27
6	0.02	0.35	0.37	8.97	0.03	0.48
7	0.11	0.01	1.21	0.52	1.39	0.48
8	0.11	0.39	0.58	0.17	0.58	0.94
9	0	1.49	2.13	6.62	0.16	0.06
10	0.09	0.05	2.42	2.97	2.93	3.22
1 st Quart	0.02	0.03	0.11	0.08	0.18	0.10
Median	0.09	0.12	0.68	0.48	0.89	0.31
3 rd Quart	0.11	0.36	2.20	3.88	5.17	1.27
IQR	0.10	0.33	2.09	3.80	4.99	1.18
Lower	-0.13	-0.46	-3.02	-5.62	-7.31	-1.67
Upper	0.25	0.85	5.34	9.59	12.65	3.04
Mean	0.08	0.29	1.02	2.11	3.48	0.78
cor MSE	0.06	0.16			2.09	0.51

Table 14 Calculations of outliers. Table shows all errors calculated from LOOCV of each two covariate model. From the 1st and 3rd quartile the inter quartile range was calculated (IQR). The upper and lower values represent the limits for outliers (Lower = 1Q - 1.5*IQR and Higher = 3Q + 1.5 * IQR) [186]. The error values in bold are outliers according to these calculations. At the bottom are the mean and corrected mean where the outliers have been removed.

	<i>SH</i>	<i>SS</i>	<i>PH</i>	<i>PS</i>	<i>HH</i>	<i>HS</i>
1	0.09	0.25	0.01	0.00	0.79	0.07
2	0.00	0.00	0.03	0.09	0.27	0.00
3	0.13	0.12	8.41	0.17	9.17	0.02
4	0.06	0.37	0.08	0.00	0.93	0.16
5	0.08	0.65	0.53	0.00	13.13	0.01
6	0.00	1.33	0.48	2.82	0.01	0.16
7	0.05	0.09	0.96	0.73	0.44	0.80
8	0.08	0.22	0.42	0.15	0.05	1.14
9	0.10	0.01	0.53	6.56	1.41	0.00
10	0.09	0.04	3.70	0.01	0.00	0.40
1 st Quart	0.04	0.03	0.07	0.00	0.04	0.01
Median	0.08	0.17	0.50	0.12	0.61	0.12
3 rd Quart	0.09	0.44	1.65	1.25	3.35	0.50
IQR	0.06	0.41	1.58	1.25	3.31	0.49
Lower	-0.01	-0.45	-1.87	-1.75	-4.35	-0.62
Upper	0.16	0.79	2.87	1.99	5.58	0.85
MSE	0.07	0.31	1.52	1.05	2.62	0.28
Cor MSE		0.19	0.38	0.14	0.49	0.18

Table 15 Calculations of outliers. Table shows all errors calculated from LOOCV of each one covariate model. From the 1st and 3rd quartile the inter quartile range was calculated (IQR). The upper and lower values represent the limits for outliers (Lower = 1Q - 1.5*IQR and Higher = 3Q + 1.5 * IQR) [186]. The error values in bold are outliers according to these calculations. At the bottom are the mean and corrected mean where the outliers have been removed.

	<i>SH</i>	<i>SS</i>	<i>PH</i>	<i>PS</i>	<i>HH</i>	<i>HS</i>
1	0.00	0.55	0.00	0.02	0.48	0.14
2	0.00	0.02	0.07	0.35	0.15	0.00
3	0.03	0.13	0.07	0.01	1.45	0.00
4	0.00	0.59	0.11	0.31	0.02	0.17
5	0.03	1.42	0.18	1.05	1.41	0.00
6	0.00	0.36	0.18	3.21	0.00	0.17
7	0.06	0.00	0.57	0.27	0.48	0.42
8	0.00	0.02	0.07	0.41	0.38	0.12
9	0.09	0.80	0.00	68.81	0.15	0.05
10	0.08	0.03	2.69	0.01	0.67	0.42
1 st Quart	0.00	0.02	0.05	0.02	0.12	0.00
Median	0.02	0.24	0.09	0.33	0.43	0.13
3 rd Quart	0.07	0.64	0.27	1.59	0.85	0.23
IQR	0.07	0.62	0.22	1.58	0.73	0.22
Lower	-0.09	-0.69	-0.24	-2.03	-0.67	-0.21
Upper	0.12	1.18	0.42	2.69	1.53	0.47
MSE	0.03	0.39	0.39	7.44	0.52	0.15
cor MSE		0.28	0.09	0.30		

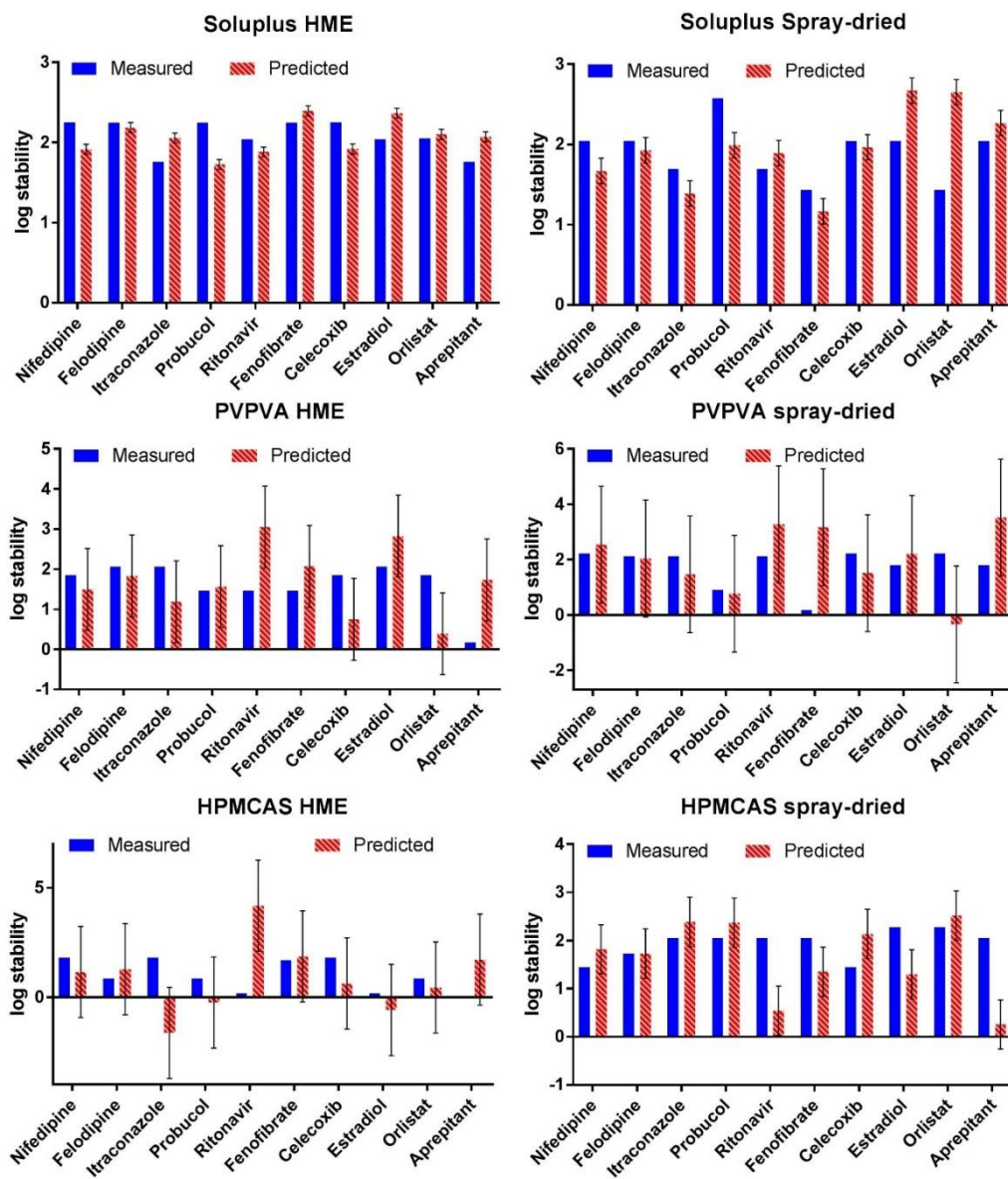


Figure 27 Leave-one-out cross validation of each of the six models. The error bars on the predicted stability is the MSE of each model.

3.3.2. Ranking

The purpose of these models is to indicate which combination of processing method and polymer carrier is most likely to give a stable solid dispersion. Thus the models are used to rank the stability of the formulations so that the best combination can be found. Figure 28 shows the ranking of formulation combinations, using the three covariate models.

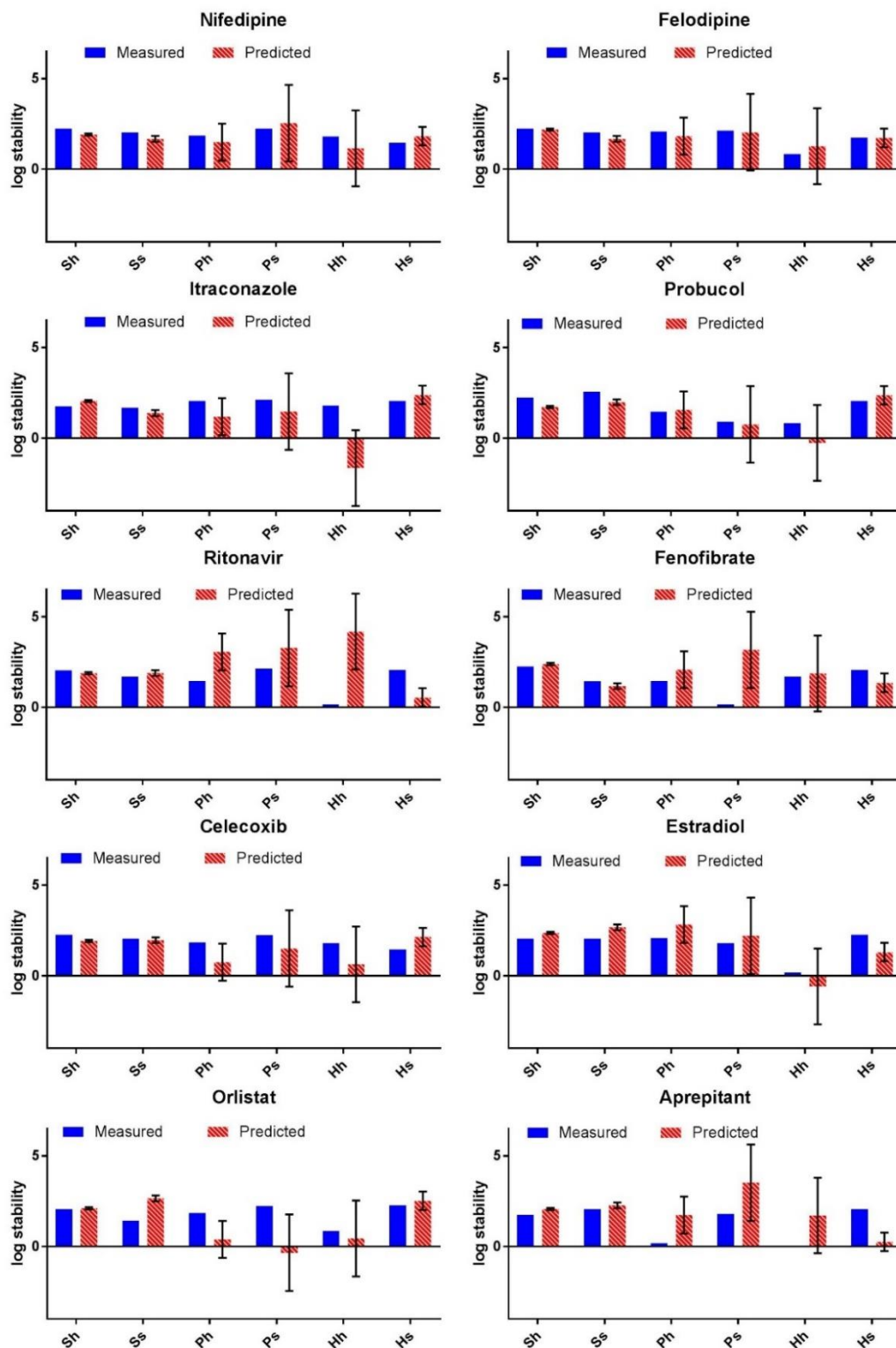


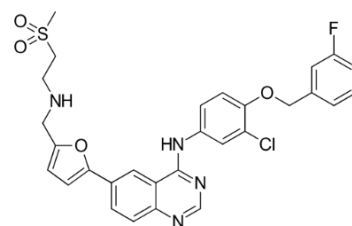
Figure 28 Ranking of formulation combinations each API and polymer-manufacturing combination using the tree covariate models. The error bars show the MSE of each model. The predicted ranking is in good agreement with the measured stability. The following abbreviations are used: SH – SOL HME, SS – SOL spray-dried, PH – PVP-VA HME, PS – PVP-VA spray-dried, HH – HPMCAS HME and HS – HPMCAS spray-dried.

3.3.3. External validation

A further external validation was performed. A recent study compared the stability of three solid dispersion formulations of lapatinib (structure Table 16), namely SOL, PVP-VA and HPMCAS. These were spray-dried at 40% w/w API and stored at 40 °C / 75% RH. It was found that the formulation containing HPMCAS was the most stable by far, staying amorphous for the six months tested while both PVP-VA and SOL formulations were crystalline by one month [187]. Using our Ss, Ps and Hs models, the stability of lapatinib formulations was predicted for each polymer. The models ranked the stability correctly both using two- and three-parameter models. For the three-parameter models the stability was less than one month for SOL and PVP-VA and more than six months for HPMCAS, reproducing perfectly the measured stability (Table 16)

Table 16. Measured [187] and predicted stability of lapatinib formulations, using one, two and three covariate models. The molecular structure of lapatinib (right).

	<i>SOL</i>	<i>PVP-VA</i>	<i>HPMCAS</i>
<i>Measured</i>	< 1 month	< 1 month	> 6 months
<i>Predicted 3</i>	19 days	0 days	220 days
<i>Predicted 2</i>	19 days	0 days	30 days
<i>Predicted 1</i>	40 days	129 days	55 days



3.4. Discussion

3.4.1. Suggested guidelines

All six models (Table 11) contain three physicochemical parameters, but no two models contain the same three, showing the complexity of the stability of solid dispersions. However, some general rules can be deduced from the six models.

The following are correlated with increased stability of the solid dispersion.

1. Increased T_g of the dispersion (measured or predicted)
2. Increased molecular flexibility of the API
3. Decreased number of H-bond donors (or Fluorine / Nitrogen atoms)

The first rule is in good agreement with previous studies. As stated above many studies have considered the correlation between T_g and solid dispersion stability [120]. This often relates to choosing a high T_g polymer in order to obtain a solid dispersion with a high T_g . Moreover, storing the solid dispersion 50°C below the T_g of the solid dispersion reduces the molecular mobility to a minimum, thus increasing the stability (reviewed in [188]). Thus for storing the solid dispersion at room temperature a solid dispersion with at least a T_g of 75 °C would be preferable.

The second rule indicates that the more flexible API molecules are, the less likely they are to adopt the correct conformation for crystallisation. In the models the parameters relating to flexibility are aromatic atom count, rotatable bonds, chiral atoms and ring count. The parameters hindering flexibility, aromatic atom count and chiral atoms, were negatively correlated with stability while the opposite was observed for rotatable bonds, which increases flexibility. Ring count was positively correlated although it would be expected to be negative, however, this parameter only occurred in the model with the lowest R^2_{adj} . Aromatic rings can interact strongly through van der Waals interactions and π - π stacking, symmetry drives interactions and intermolecular bonding, while ring structures have been showed to cause dense packing structure in crystals, all of which can drive crystallisation [5].

The correlation between flexibility, especially the number of rotatable bonds, and amorphous stability or crystallisation tendency has been noted in several computational studies [43,100,101,105,176,189,190]. A recent classification of crystallisation tendencies according to flexibility and size was proposed from reviewing published datasets and models in this area. The classification indicates decreasing crystallisation inclination is correlated with increased molecular weight and flexibility (rotatable bonds) as can be seen in Table 17 [191].

A predictive model using a partial least squares projection to latent structure discriminant analysis (PLS DA) was built based on experimentally determined glass-forming ability of 16 drugs. The model showed a negative correlation between glass forming ability and number of benzene rings, but a positive correlation to branching of the carbon skeleton, molecular symmetry and distribution of electronegative atoms in the API [47]. Another study found that APIs with high molecular weight and flexible structures were more likely to be glass formers than small rigid molecules [43]. Several models using calculated, predicted and measured parameters have been built to predict the amorphous stability of APIs. In models containing rotatable bonds these were positively correlated with stability whilst aliphatic rings were negatively correlated [100]. These studies only examined the API alone and not in a solid dispersion as here, moreover two of them only look at glass-forming ability, not stability. However, they show that flexibility and structural complexity of the API is strongly connected with its crystallisation tendency, which was highlighted by four flexibility/complexity (nChir) parameters in the models above. It can, therefore be concluded that in regards to flexibility of the API, the same applies to glass forming ability of API on its own and the stability of the amorphous form in a solid dispersion.

Table 17 A recent classification for the crystallisation tendencies of APIs based on size and flexibility. The five classes represent how easily an API crystallises [191].

<i>nRot /MW</i>	<i>< 300 g/mol</i>	<i>> 300 g/mol</i>
≤ 3	I Easy	III Moderate
4-6	II Easy to moderate	IV Moderate to difficult
> 6	IV Moderate to difficult	V Difficult

The first two rules are intuitively expected whereas the third one, less so. H-bonds are an important factor to stabilise solid dispersion as discussed before. Several studies have found hydrogen bonding to be important for the glass forming ability or stability of glasses [46,100,192]. Furthermore, a positive correlation has previously been found between well-distributed electronegative atoms and glass forming ability, which may be due to the H-bonding ability of such atoms [5,47]. The number of HBA was identified as a good predictor of stable glass formers in combination with the absolute Huckel pi atomic charge of carbon atoms [46]. The atomic mass-weighted third-order R autocorrelation index (R3m) molecular descriptor has been found to be the most significant predictor of miscibility in PVP-VA solid dispersion. This was based on experimental data from 12 APIs. The R3m is a complicated molecular descriptor but is associated with electronegative atoms and their positions in the molecule [57]. None of these studies considered the stability of solid dispersions in the context of H-bonding. Here, however, H-bond donors and acceptors, as well as number of nitrogen atoms, are shown to be negatively correlated with stability in all cases but for N count in the Ps model. For the three polymers studied here only HPMCAS has any H-bond donors, but all have H-bond acceptors. However, even if there are H-bond acceptors available in the carrier polymer that does not necessarily mean that API-polymer H-bonds will actually be formed. Instead, these H-bond donors/acceptors in the APIs could lead to API-API bonding or API-water H-bonding, both of which lead to decreased stability and more rapid crystallisation.

These proposed rules are not intended to be absolute but to suggest guidelines, and, due to the complexity of these systems, exceptions must be expected. *E.g.*, even though increased T_g is correlated with increased stability, SOL HME, solid dispersions were the most stable of the HME formulations overall, despite SOL having the lowest T_g out of the three polymers. Similar examples can be found for the molecular structure flexibility. If the two extremes are compared, estradiol is the most rigid API in the study with three aliphatic rings, one aromatic ring and no rotatable bonds. Orlistat is the other extreme with only one aliphatic ring, no aromatic rings and 23 rotatable bonds. If solid dispersion stability was only dependent on API flexibility orlistat would be expected to be much more stable than estradiol in all formulations. However, this is not the case, orlistat is more stable in two formulations PVP-VA spray-dried (52 weeks vs 26 weeks) and HPMCAS HME (4 weeks vs 1 week). In two instances, the stability is the same for the two drugs in comparable formulations: SOL HME and HPMCAS spray-dried, while in the latter two formulations estradiol is more stable than orlistat.

3.4.2. Predictability

The MSE ranges from minimal in Sh model to very large in the PVP-VA models in particular. If the predicted values are compared to the measured ones they are overall mostly in good agreement, Figure 29. However, especially for the PVP-VA and Hh models a few APIs are very poorly predicted, such as ritonavir in both Ph and Hh or fenofibrate in Ps. These, during the LOOCV process, were predicted using models that varied from the main models. The LOOCV models for these drugs used parameters for which the APIs had extreme values, differing greatly from the rest, such as for ritonavir both Ph and Hh models contained tPSA which in both cases ritonavir has the highest value by far or 145 compared to most other well under 100. This difference in chosen models in LOOCV is mostly due to the relatively small dataset, with more data the models are more robust and less likely to be as affected by leaving out a single value. These poor predictions for a single or a few compounds causes large increase in the MSE as can be observed in Figure 29.

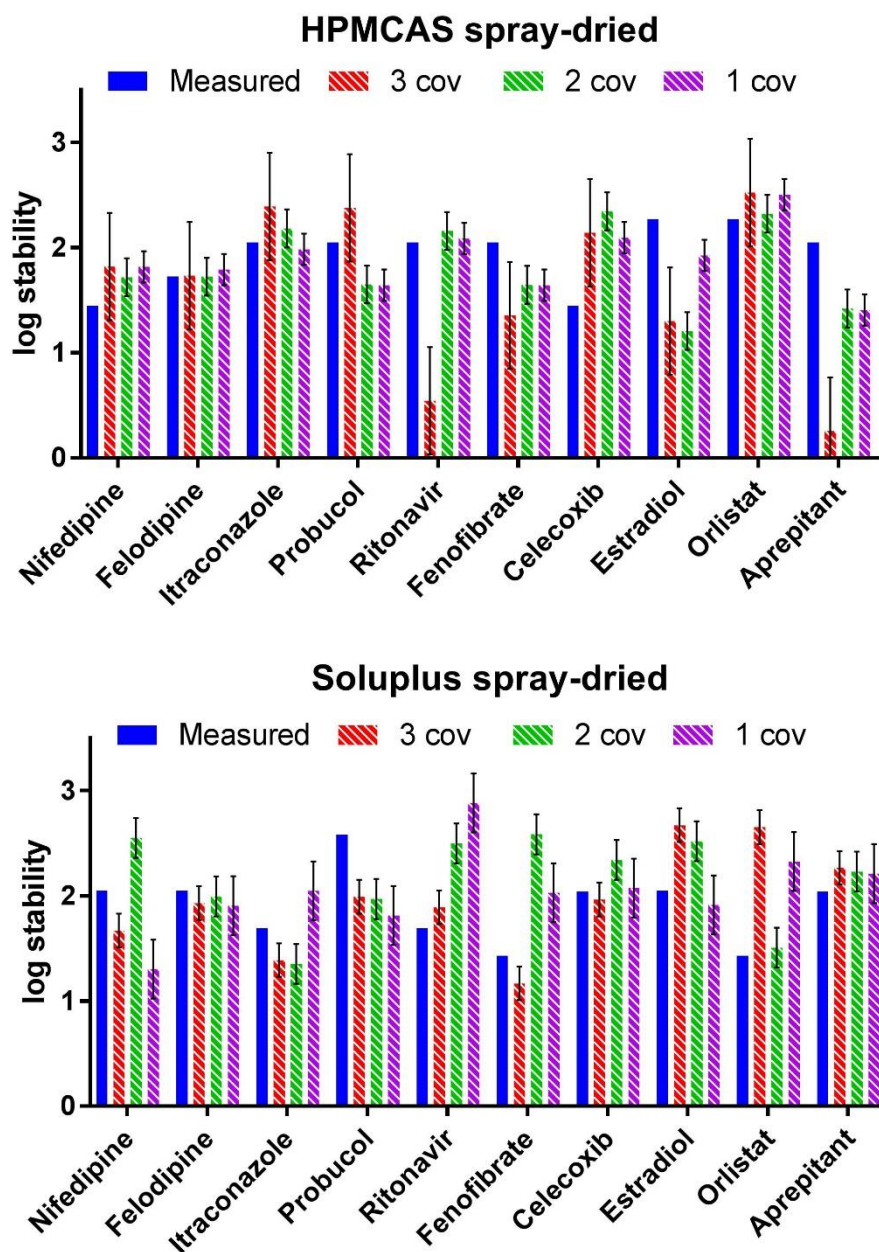


Figure 29 comparing the predicted values and MSE values for three, two and one covariate models. On the top, Hs models and Ss on the bottom. For Hs the MSE values decrease with decreasing covariates while the opposite is observed for Ss. When the predicted values for stability are compared with the measured ones they are generally in good agreement for all covariate models. Exceptions are for example three covariate Hs model predicts stability for ritonavir and aprepitant very poorly. Similarly, for Ss, Ritonavir and Orlistat are poorly predicted with one and three covariate models, respectively.

Although the MSE is used here to describe the accuracy of the model predictions, presented as error bars in graphs, it is not directly connected with the models. When performing the LOOCV the whole modelling process is repeated each time an API is left out, including choosing the best parameters. Thus the models produced in the LOOCV and the calculated MSE will remain the same irrespective of the model produced using the whole dataset. For instance, a Sh model containing nF showed higher R^2_{adj} than without nF. Although the two models (with and without nF) had different R^2_{adj} the MSE was the same. This is because the MSE explains the variation in the dataset rather than the confidence level of the predictions of the model itself. Therefore, if there is a very strong correlation that is seen for all the data-points (APIs) it is likely that the same physicochemical properties would be picked in all the LOOCV models, and thus give a more accurate prediction and lower MSE. Furthermore, increasing the number of observations, decreases the effect each one has on the MSE.

As mentioned above, a Sh model containing nF showed a better R^2_{adj} than the one used here. As only two out of ten APIs contain fluorine atoms it is hard to draw conclusions about the effect of fluorine on the solid dispersion stability. However, with more fluorine containing APIs this trend could be confirmed or rejected.

3.4.3. External validation

When comparing the models using one, two and three covariates the following can be observed, as expected the R^2_{adj} falls when number of covariates is decreased. However, when looking at the MSE this decreases in four cases out of six. The lower MSE might suggest that the higher number of covariates is over-fitting the model. In order to test the models against over-fitting an external validation is needed.

The three covariate models correctly predicted the stability of all three formulations. While the two covariate models gave a correct ranking of the formulations the predicted stability of the Hs formulation was much shorter than observed. However, as noted before these models are not intended to predict the

stability as such but rather correctly predict which formulation, combination of polymer and manufacturing method, will be most stable. A correct ranking of formulation stability is therefore the goal. The one covariate models were not able to correctly rank the formulation stability.

The external validation shows that the models are nevertheless able to correctly predict the ranking of formulations containing a novel drug. Moreover, the predicted stability was in very good agreement with the measured stability, thus showing that despite a relatively small dataset, in terms of statistics, the models can be applied on real data to give a true ranking of formulations, which is promising as a support tool in solid dispersion formulation development.

3.5. Conclusions

Predictive statistical models were built for six combinations of manufacturing method and polymer carriers. These show the potential of using such models in formulation development. Furthermore, none of the models are identical which indicates the complexity of these systems. Three rules that could guide formulation development can be deduced from the models. Increased stability is correlated with increased T_g of solid dispersion, as are decreased number of H-bond donors and increased molecular flexibility of the API. Despite high MSE, LOOCV showed good predictability which was confirmed in an external validation of three formulations.

The auspicious results presented here give hope that these types of knowledge based computational support tools can be used in formulation development in the near future. The models built here could in fact, as there are none currently available, be made open-source knowledge based tools, to be used for guiding early formulation development, as they are. However, in order for these models to be used for that purpose more reliably, further data would be needed to improve their predictability and robustness. Furthermore, a more expansive external validation would diminish the risk of model overfitting.

Chapter 4 – Conclusions

4.1. Summary

Despite substantial effort in research studies the correlation between physical stability of solid dispersions and factors such as the carrier, manufacturing method and properties of the API have not been fully elucidated. This is partly due to limited number of formulations investigated in each study. Here an extensive dataset of 60 formulations was used in order to shed a light on these correlations.

From a broad range of neutral, poorly soluble APIs, ten chemically diverse APIs were chosen. These were equally divided between class II and III GFA, non-stable and stable glass formers. Three polymers with varying properties that represent carriers used in marketed solid dispersions were used as well as the two manufacturing methods most commonly used in the industry. In order to limit the number of variables affecting the stability, all formulations that were produced with the same method, were done so using the same conditions. The only two exceptions were itraconazole spray-dried which was made using a different solvent and orlistat HME which was extruded directly without circulation inside the HME. All formulations were subjected to an accelerated stability study at 40 °C/75 % RH for a minimum of six months or up to one year. During the stability study, spray-dried powders containing either SOL or PVP-VA transformed into glass and crystallised. This transformation was expected to be due to water uptake, as the phenomenon was not seen in HPMCAS, the least hygroscopic of the three polymers. To begin with all samples were analysed for signs of crystallisation using three analytical techniques, DSC, XRPD and PLM. Once it was observed that PLM was by far the most sensitive method analysis with DSC and XRPD were discontinued. This allowed for much faster and more sensitive analysis of stability for the 60 formulations produced.

Results from the stability study showed substantial difference in stability between manufacturing methods, carrier polymers and APIs. However, no clear correlations were observed by simple review of the data. In general, PVP-VA and HPMCAS, spray-dried formulations seemed to be more stable than the HME ones. The opposite was observed for formulations containing SOL. Furthermore, when comparing the stability of formulations containing stable and non-stable glass formers no difference was observed.

A range of calculated and predicted physicochemical properties of the APIs were obtained using Instant JChem. These properties described size, surface area, H-bond donors and acceptors, lipophilicity, flexibility and complexity, and more. Furthermore, the measured and calculated T_g of the formulations was included. All of these factors along with the measured stability of each formulation were used to build multiple linear regression models. Each model included data from one combination of polymer and manufacturing method, which gave six models in total. Due to the relatively limited number of observations (but many more than has previously been achieved) each model was built on, ten, the number of covariates was constrained to maximum of three. One and two covariate models were also made. Underlining the complexity of the systems, seen in the experimental results, no two models contained all the same covariates

.

A leave one out cross validation was performed on each model and mean square error calculated from the predicted values. All three covariate models had excellent R^2_{adj} or ≥ 0.8 . The MSE in contrast was high for three models, HPMCAS HME, PVP-VA HME and PVP-VA spray-dried. The MSE decreased for these models when using only two or one covariates. The MSE was substantially higher when outliers were not disregarded and in some instances other trends were seen, such as for Ps one covariate model the MSE was significantly higher than for two and three covariate models. The observed decrease in MSE which was seen in some of the models when the number of covariates was reduced, could indicate over-fitting of the model. An external validation was performed for all spray-dried models using data from the literature. It was conducted on one, two and three covariate models.

The predicted stability from the three covariate models was in excellent agreement with the measured values. For the two covariate models, the ranking of formulations was correct but prediction of stability worse than the three covariate models. The one covariate models, however, failed to predict the ranking of formulation stability. It is interesting to note the accurate predictions, especially for the three covariate models, despite the external formulations containing 40 % drug content compared to 10 % used here.

Three rules were deducted from the models. These are intended as general guidelines in the formulation development of solid dispersions. Increased stability of solid dispersions is correlated with increased T_g of the solid dispersion, increased flexibility of the API molecule and decreased number of H-bond donors in the API molecule.

Overall the models showed promising results, especially in the external validation. Although more data needs to be added to increase the robustness these are proposed to be a good proof of concept.

4.2. Critique

As with any work there are limitations that must be recognised. Here the dataset was relatively limited, due to the constraint in experimental time within a PhD, to 10 neutral poorly soluble drugs. Most NCE are poorly soluble and although not all are neutral, at least 67% of the APIs in marketed solid dispersions are, see table 3 in Chapter 2. Neutral APIs have fewer formulation routes available to improve solubility compared to charged species, where salt formation can be used, for example. This is indeed part of the rationale behind choosing these for the dataset. Additionally, with a limited dataset, constraints must be used for choosing the APIs in order to maximise the chance of finding suitable correlations.

Furthermore, to keep the variables at a minimum there was no optimisation of the manufacturing methods. Thus for some APIs the manufacturing conditions, such as temperature, may be suboptimal. This could result in a less stable formulation than a formulation made using an optimised method. However, if each method had been optimised for each formulations direct comparisons could not have been made.

Finally, although the PLM was the most sensitive of the methods used here, it is more subjective than more quantitative methods, such as DSC and XRPD. However, as discussed before, the risk of misclassifying a crystal was minimised using well-defined criteria when analysing samples.

Other experiments that could have been included in this study and would have added valuable information are for example Raman spectroscopy or dissolution. As stated before, specific interactions between polymer and API can have positive effect on stability of solid dispersions. Methods such as Raman, IR or NMR can be used to look at interactions such as H-bonding.

Dissolution study of formulations would also be a beneficial addition to this work as a successful solid dispersion formulation requires both stability and suitable dissolution. Furthermore, although a very difficult task, a model that could predict both stability and dissolution would be an extremely desirable and helpful tool in the formulation development of solid dispersions.

Although from a pharmaceutical point of view the dataset presented here is very expansive, from a statistical point of view it is limited. Therefore, ideally only one, or two at most, covariates should be used in a model to minimise the risk of overfitting. Here one, two and three covariate models were made. An external validation was performed on half the models (spray-drying) which, showed excellent predictability and thus increasing the confidence of the three covariate models not being overfitted.

Furthermore, using a training set, and a test set, is more desirable than a leave-one-out cross validation. However, that would require a substantially larger dataset. A test set, similar to an external dataset contains new data, which is not used to build the model. Thus giving a more accurate indication of the predictability and possible overfitting of the model. Here the best approach would be to use an external dataset as a second validation method, which was done. However, thorough search of the literature only retrieved one study which could be used for this purpose. As previously stated most studies only investigate a very limited number of formulations. The APIs used here are well studied and thus the subject of many of the available literature. Proprietary information was a problem in some cases, as no molecular structure was given for the API used. Additionally, finding a study using two or more of the polymers used here (HPMCAS, PVP-VA or SOL) or both methods was very rare. Furthermore, only a limited number of studies investigate long-time stability of solid dispersions. Stability conditions, analysing method and drug loading were not regarded in the search.

With this limited dataset, it was nevertheless possible to demonstrate the potential of such a model and their usefulness in the formulation development.

4.3. Implications for formulation development

In conclusion, this work has provided important insight into the complex correlations between the physical stability of amorphous solid dispersions and factors such as manufacturing method, carrier and properties of the API. Due to the expansive number of formulations studied here, which is far greater than previously published in the literature in a single study, more general conclusions can be drawn about these correlations. Furthermore, using statistical analysis of the results showed correlations that would otherwise have been difficult to detect. From this analysis and model making, general guidelines could be deduced which can be a valuable help in formulation development of solid dispersions. Importantly, this work has further established a foundation for future work in this field. Specifically, in using statistical modelling in solid dispersion formulation development.

The experimental data obtained here underlines the complexity of solid dispersion physical stability. This has been widely studied but not resulted in any general agreement or conclusions observed recurrently. Despite the extensive number of formulations investigated here, few clear correlations could be observed between manufacturing method, polymer carrier, APIs and the physical stability. Mainly the stability seemed to be dependent on all three factors. Thus no single polymer or manufacturing method was observed to give substantially more stable formulations than the rest. However, when comparing combinations of manufacturing method and polymer it was observed that for SOL hot melt extrusion generally gave more stable formulations compared to those prepared with a spray-dryer. The opposite was observed for both HPMCAS and PVP-VA, using a spray-dryer better stabilised the formulations compared to the HME. These trends have not been observed previously. Furthermore, unexpectedly, it was found that there was no difference in stability of formulations containing stable and non-stable glass former APIs.

Although the models were built on a limited dataset of ten observations each, they show good predictability. Taking into account the complexity of these systems, which is further underlined with all the models containing different properties, this should be considered a great success. Although no two models built were identical there were similarities between the physicochemical properties chosen in the modelling process for each of the models. These described the flexibility and complexity of the API molecules, the H-bonding potential in the API and the glass temperature of the solid dispersion. These were used to make three rules that can be used as general guidelines in the formulation development of solid dispersions. The rules state that increased stability is linked with increased T_g and API flexibility but decreased H-bond donors. These guidelines are general, not constrained to a polymer or method. However, these are based on the formulations made here and therefore not necessarily applicable to formulations prepared with other polymers or manufacturing methods. Although, due to complete lack of guidelines, based on experimental data, available these can greatly impact the formulation development of solid dispersions.

4.4. Future work

Due to limitations in time and access to some instruments the dataset was restricted to 60 formulations. This is a considerably larger experimental dataset than has been seen in the literature before. However, in order to get more general conclusions about stability of solid dispersions this dataset could be expanded.

When choosing the APIs for this work it was decided to limit the work to neutral APIs from GFA class II and III. Expansion of the dataset to include GFA class I APIs as well as acidic and basic APIs would give a more general overview of solid dispersion stability. Furthermore, it would be beneficial to include more, commonly used polymers such as HPMC, PVP or Eudragit.

Although the models show promising results, more data is needed to create more robust models. Further data would minimise the effect of leaving out a point in LOOCV or possibly allow for a training and test set validation.

Confidence interval (CI) calculations were done in order to assess the number of APIs needed for a model giving reliable predictions, using Equation 6. The 95 % CI for the SH, SS and HS models, as is, with ten observations is less than one day. For PH, adding 10 observations, total 20 APIs, would give an acceptable 34 days margin, that is $\hat{y} \pm 34$ days, where $\hat{y}$ is the predicted stability in days. Bringing the total number of APIs up to 30 would give a margin of 30 days. The three covariate models for PS and HH have the highest MSE and poorest predictability. Even if 20 APIs were added to the dataset the margin is too high for the predictions to be reliable (>1000 days). However, it can be assumed that with more data, possibly new, stronger correlations could appear that give stronger models. New drugs to add to the dataset should have a wide range of logP values to confirm if this is a good covariate for the HH model. They should also have a range of aromatic atom count for the PS model.

$$\hat{y} \pm t_{\frac{\alpha}{2}, n-(k+1)} \sqrt{\sigma^2 x_i' (X'X)^{-1} x_i}$$

Equation 6 Confidence interval for predicted value $\hat{y}$, in multiple linear regression. For a 95% confidence interval the α is 0.05, k is the number of covariates in the model, σ the variance or MSE, n is the sample size, X is the matrix of covariates and x the covariates for a certain observation.

In order to best evaluate the predictive power of the models and test for overfitting of models, external validations should be used. Further validation of the models should be carried out, using external datasets to verify their efficacy.

Finally, more APIs that contain fluorine atoms should be included. A negative correlation seemed to be present between F atoms in APIs and stability. However only two out of ten APIs used here, contained F atoms. To confirm this effect on stability more APIs containing F atoms should be tested.

5 - References

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6 – Appendices

6.1. Additional Experimental Data

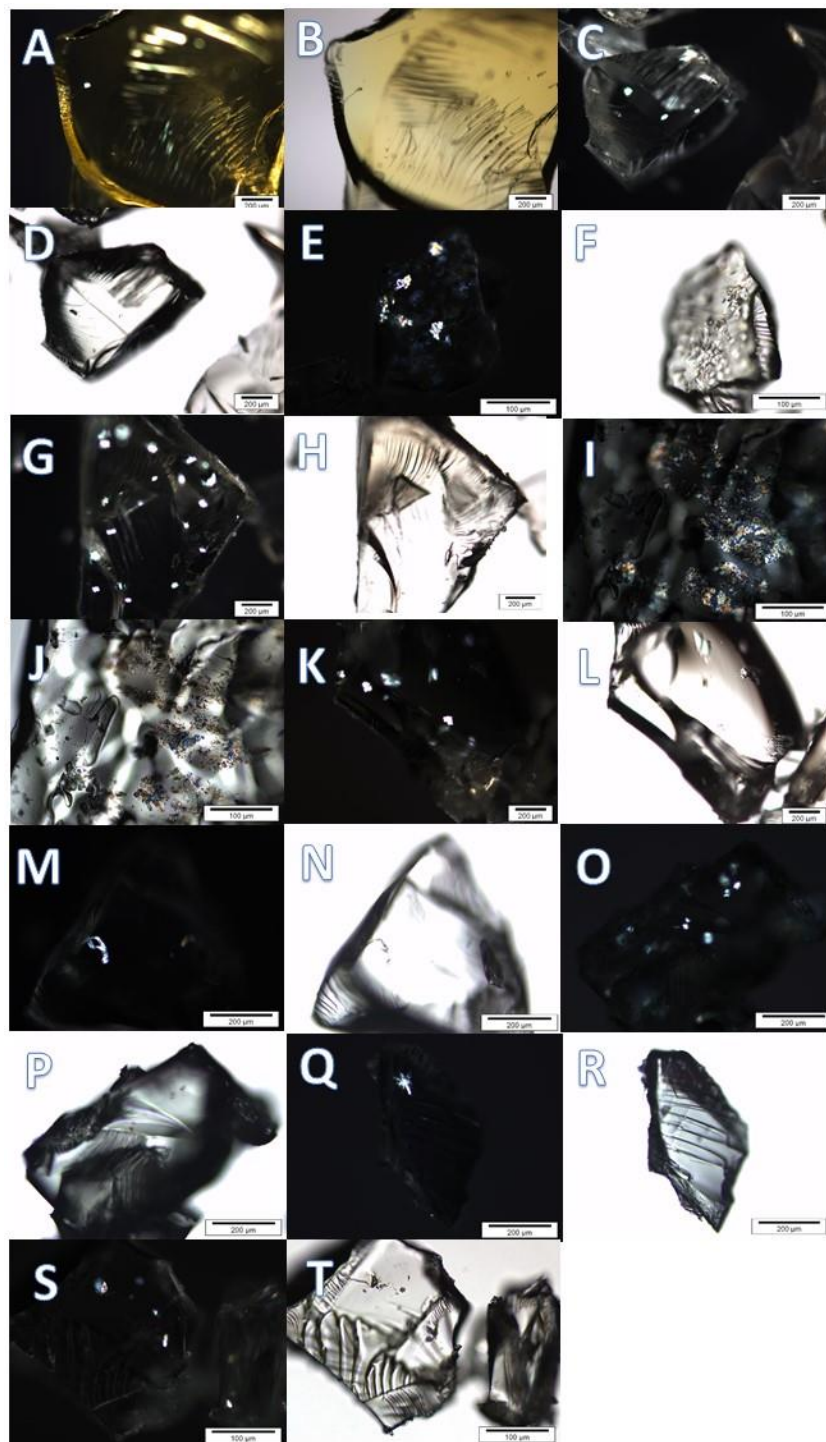


Figure 30 The point of onset of crystallisation (first time-point where crystals were observed) for each SOL HME formulation. Each picture is showed twice, once under polarised light and under normal light. A) and B) contain nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

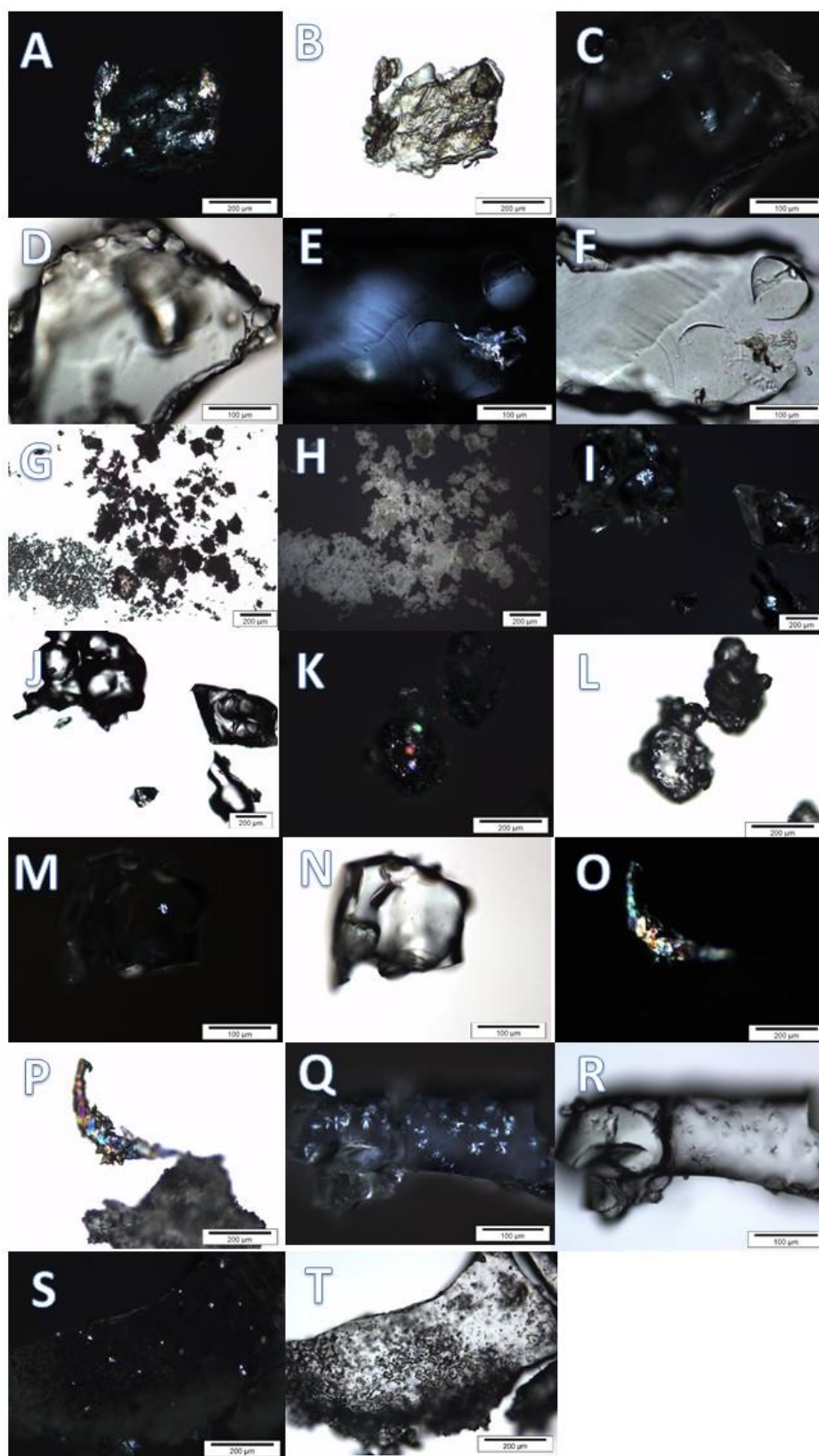


Figure 31 The point of onset of crystallisation (first time-point where crystals were observed) for each SOL spray-dried formulation. Each picture is showed twice, once under polarised light and under normal light. A) and B) contain nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

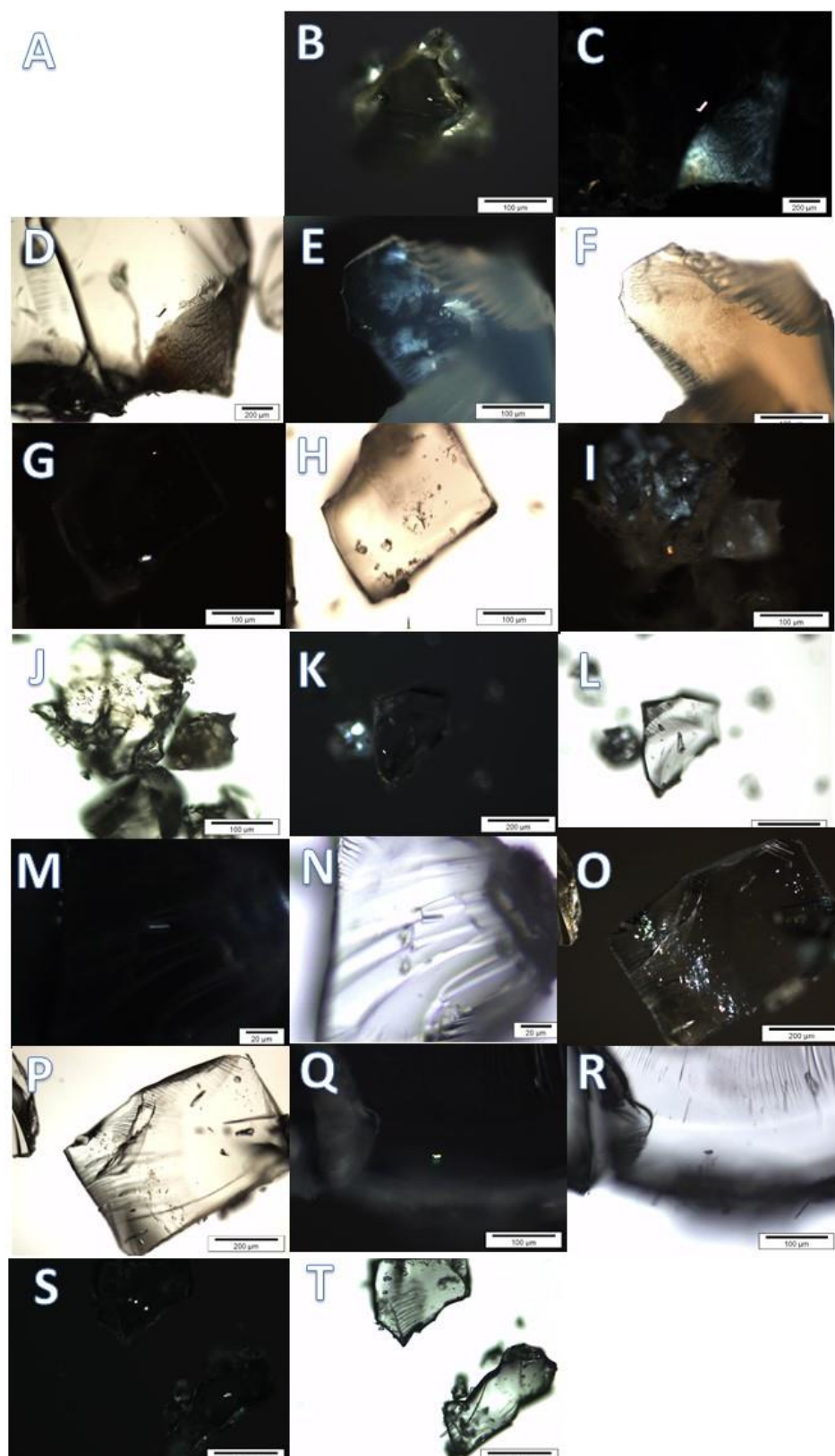


Figure 32 The point of onset of crystallisation (first time-point where crystals were observed) for each PVP-VA HME formulation. Each picture is showed twice, once under polarised light and under normal light. A) the correspondent picture to B) is not available. B) contains nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

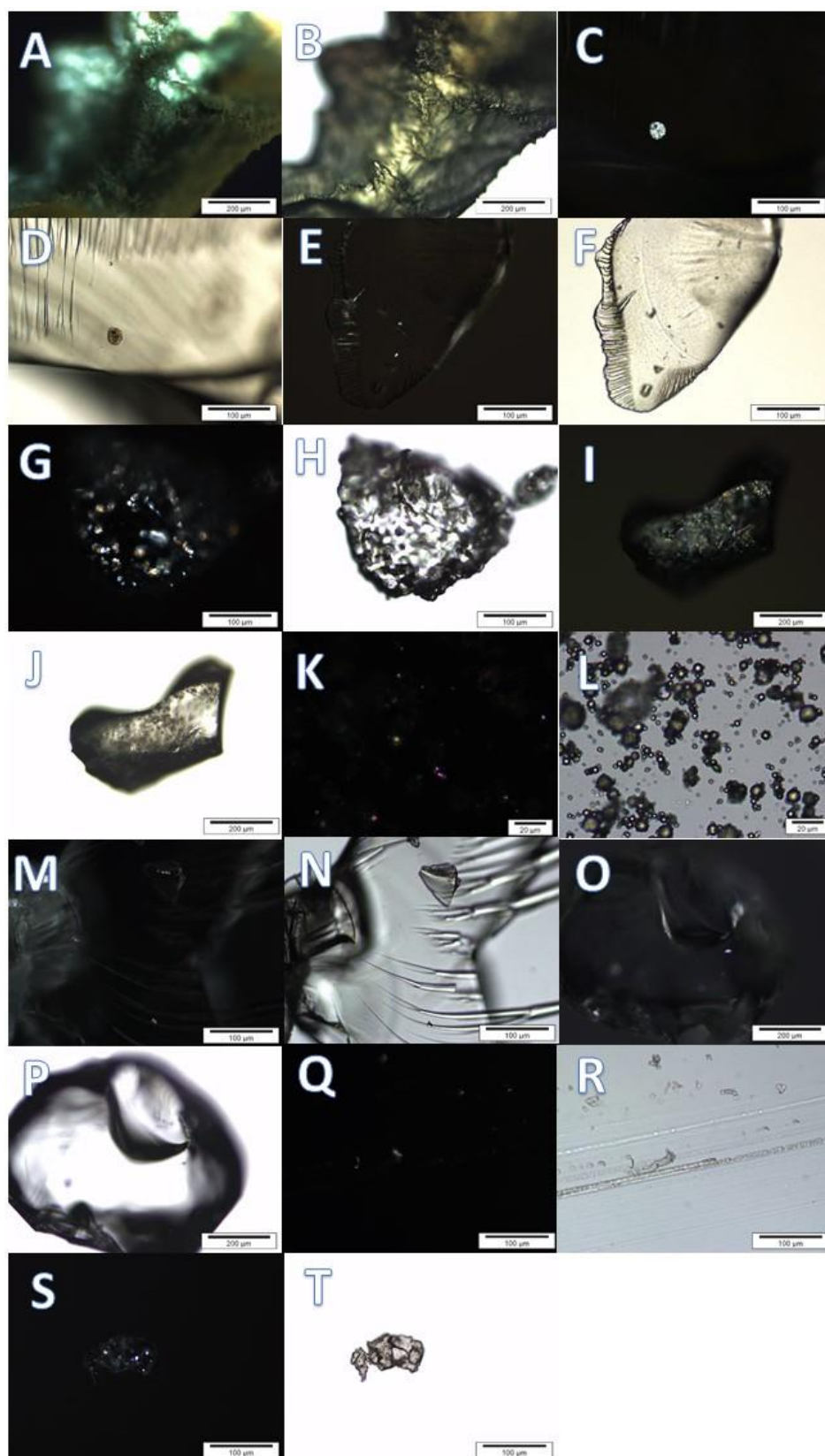


Figure 33 The point of onset of crystallisation (first time-point where crystals were observed) for each PVP-VA spray-dried formulation. Each picture is showed twice, once under polarised light and under normal light. A) and B) contain nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

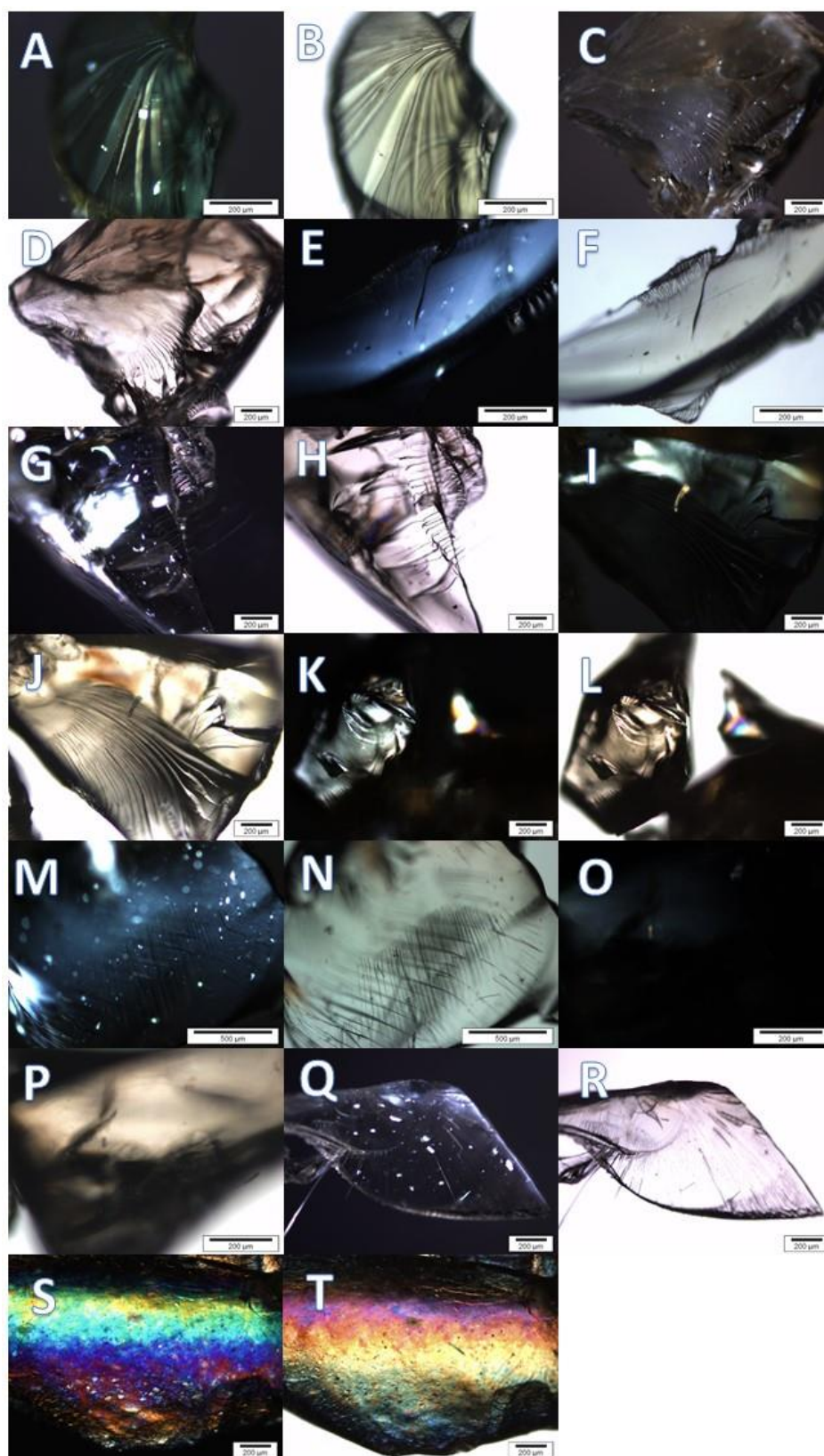


Figure 34 The point of onset of crystallisation (first time-point where crystals were observed) for each HPMCAS HME formulation. Each picture is showed twice, once under polarised light and under normal light. A) and B) contain nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

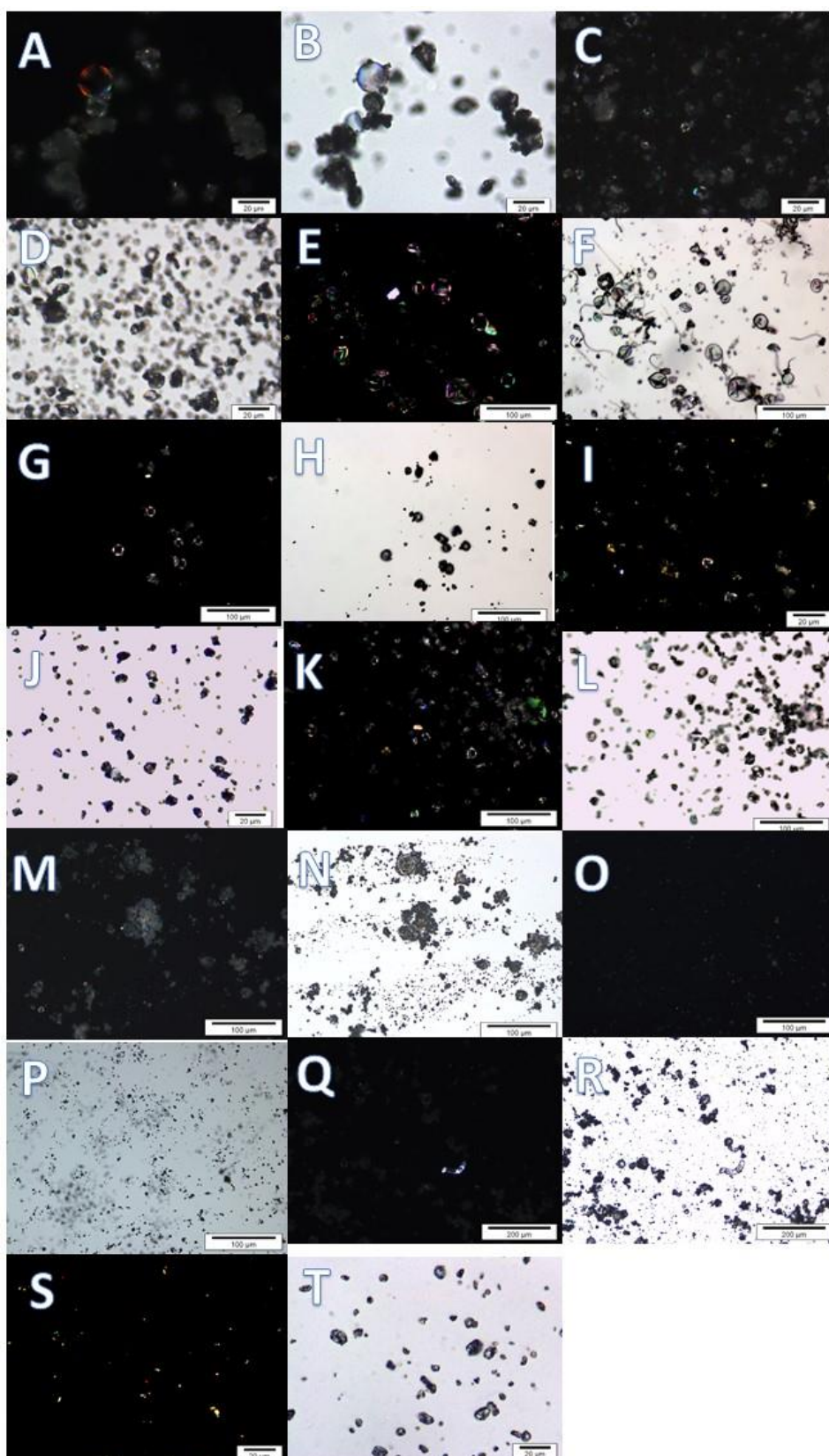


Figure 35 The point of onset of crystallisation (first time-point where crystals were observed) for each HPMCAS spray-dried formulation. Each picture is showed twice, once under polarised light and under normal light. A) and B) contain nifedipine, C) and D) felodipine, E) and F) contains itraconazole, G) and H) probucol, I) and J) ritonavir, K) and L) contains fenofibrate, M) and N) contains celecoxib, O) and P) estradiol, Q) and R) contains orlistat and S) and T) contains aprepitant

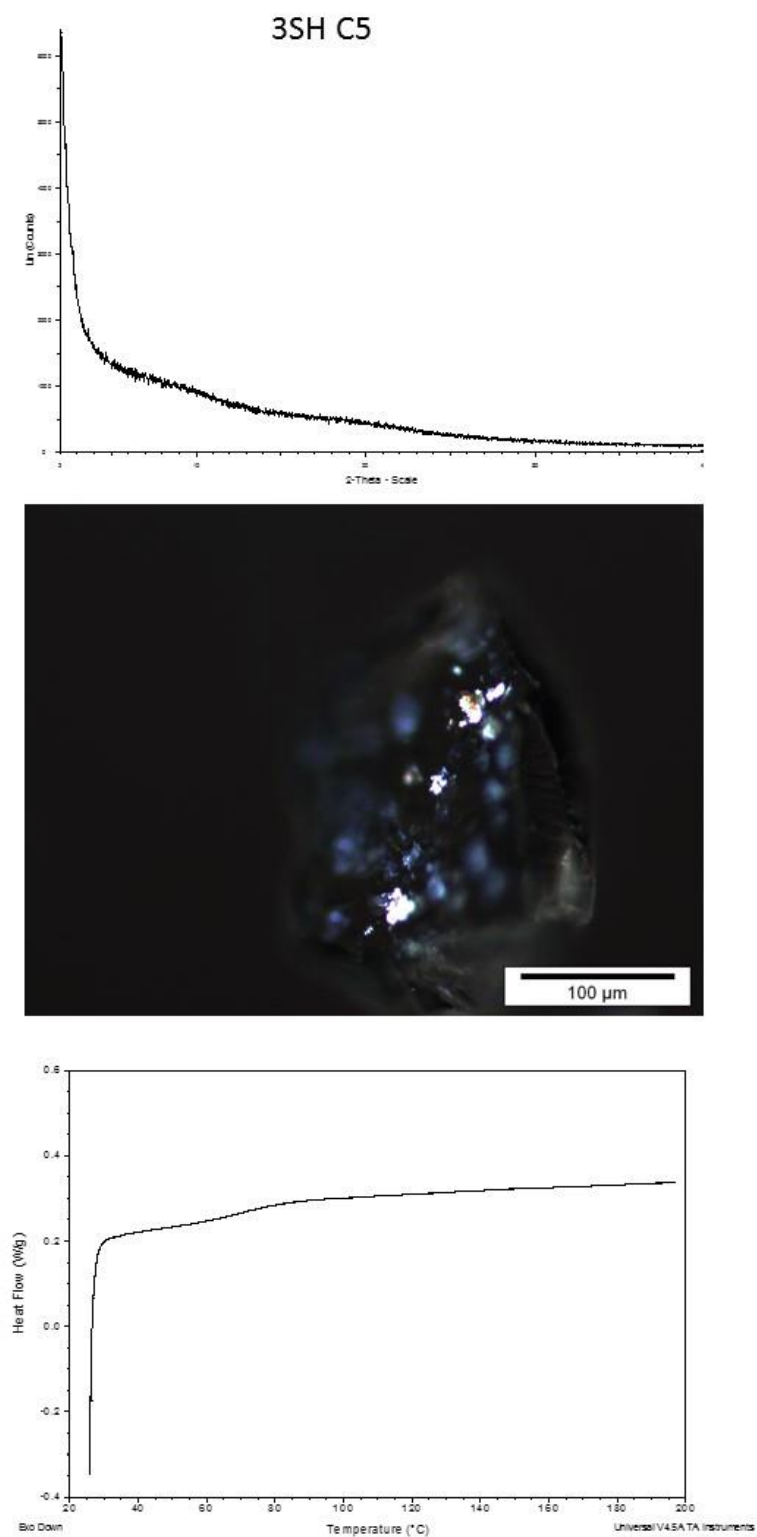


Figure 36 Formulation 3Sh at 4 months , measured with XRPD (top), PLM (middle) and DSC (bottom)

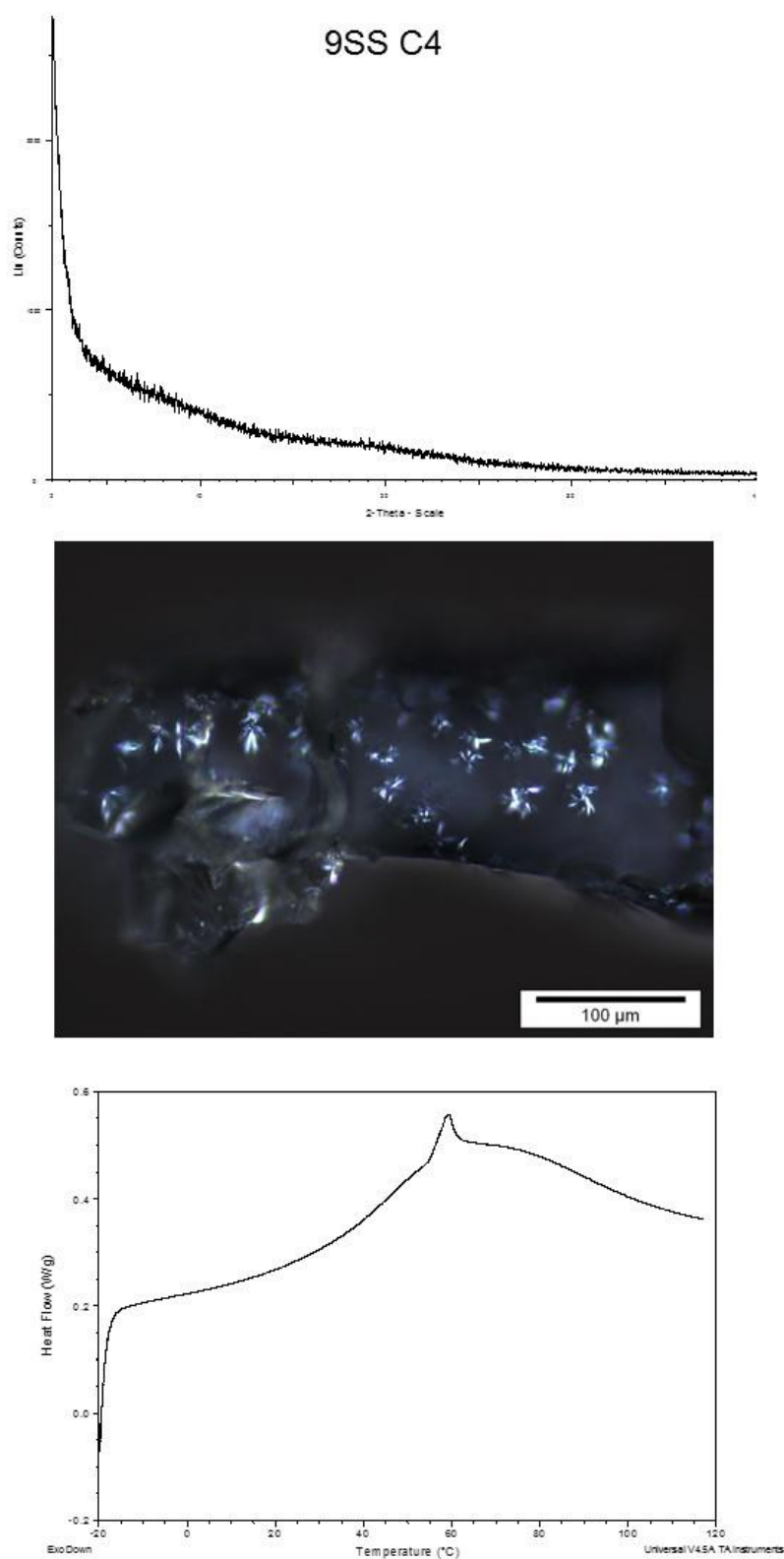


Figure 37 Formulation 9SSs at 2 months , measured with XRPD (top), PLM (middle) and DSC (bottom)

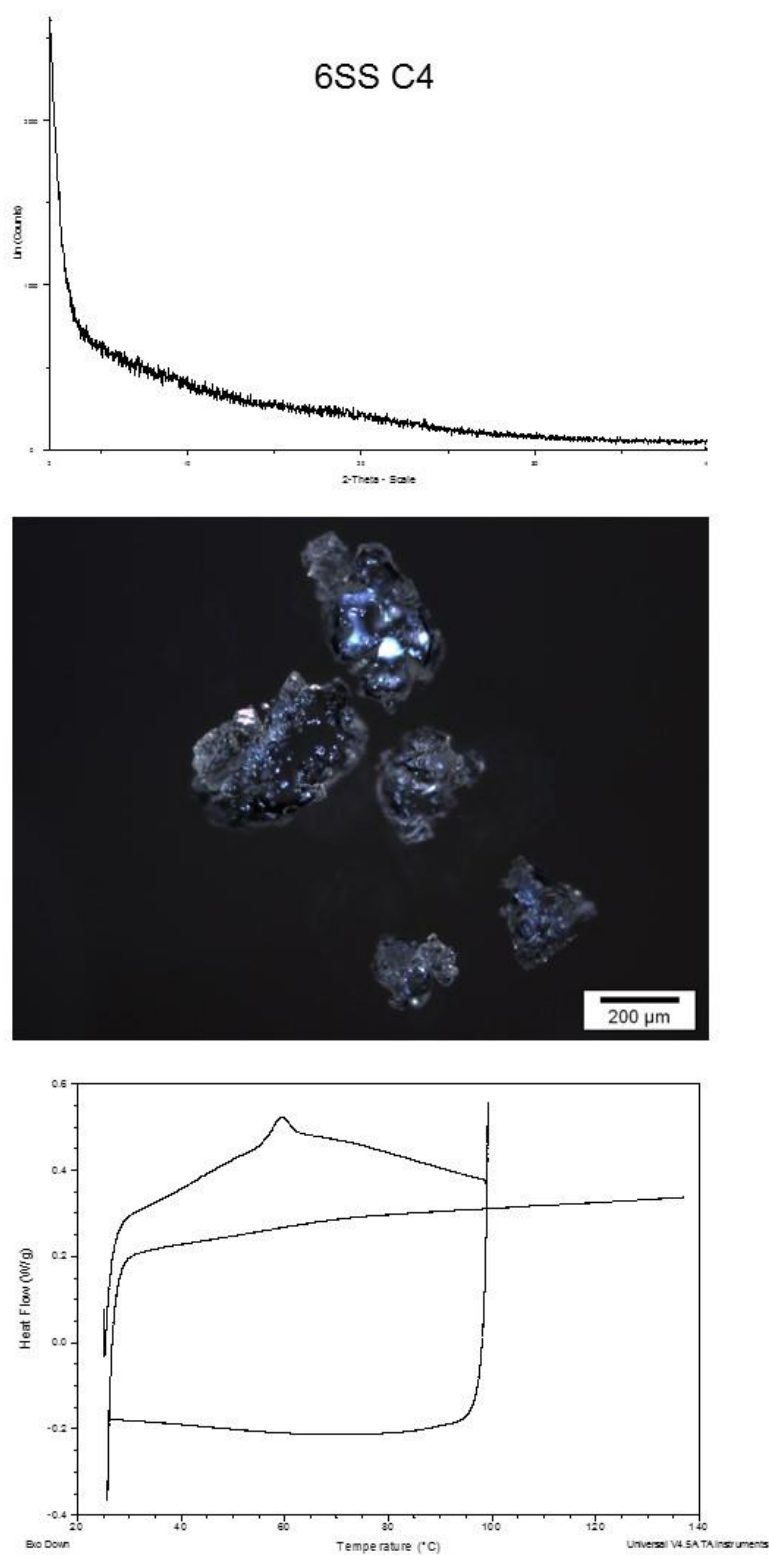


Figure 38 Formulation 6SSs at 2 months , measured with XRPD (top), PLM (middle) and DSC (bottom). A melting peak is observed during first heat ramp but not on the second one.

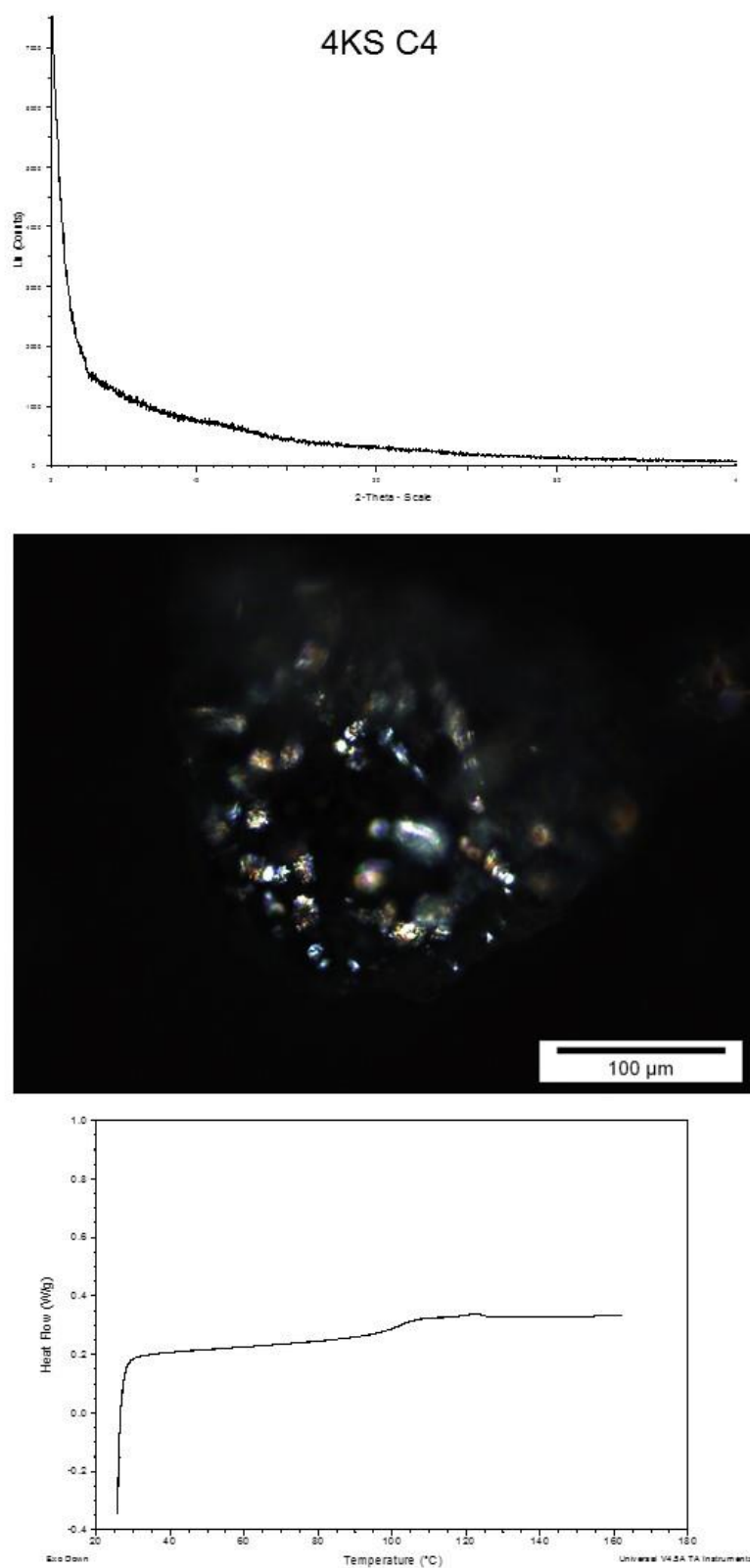


Figure 39 Formulation 4Ps at 2 months , measured with XRPD (top), PLM (middle) and DSC (bottom)

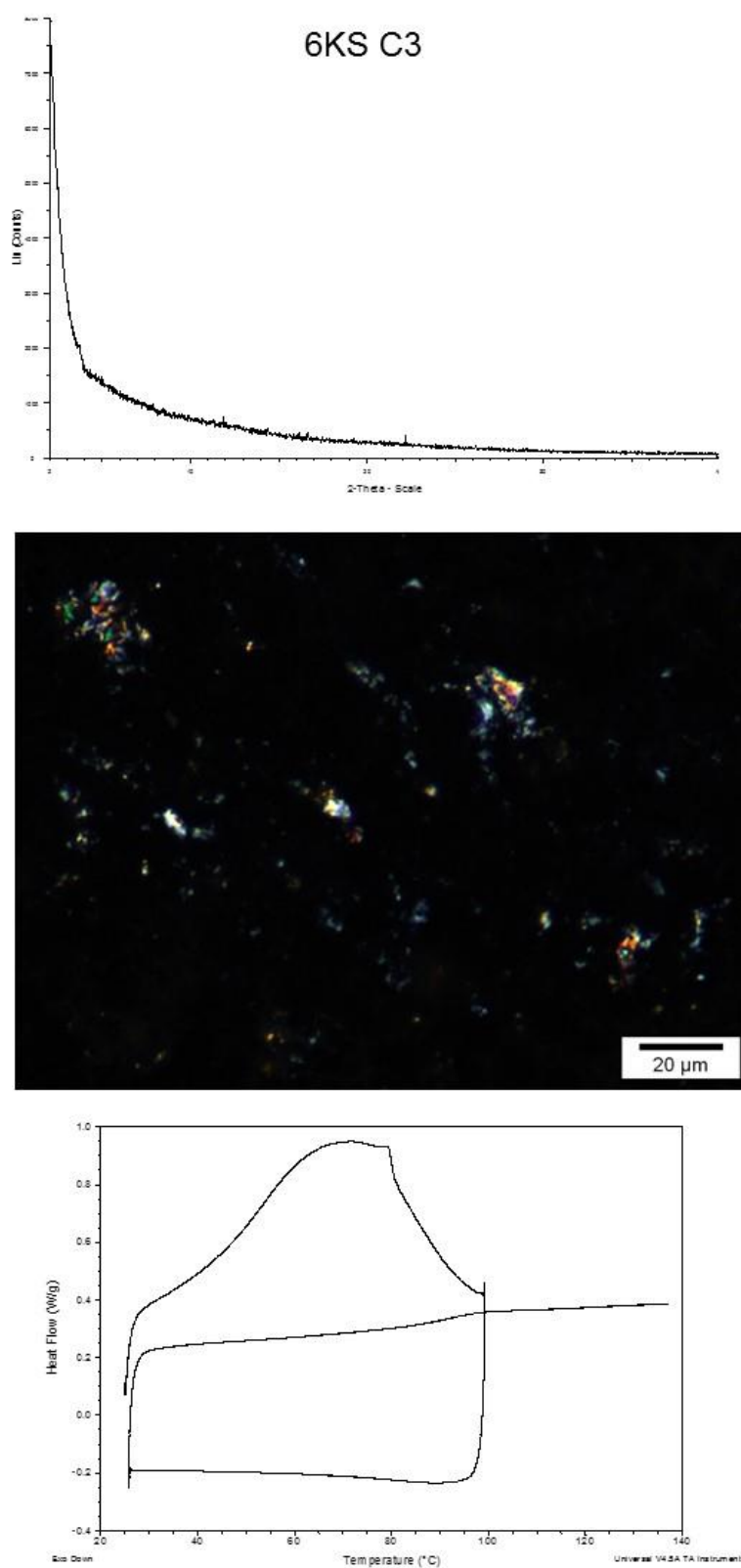


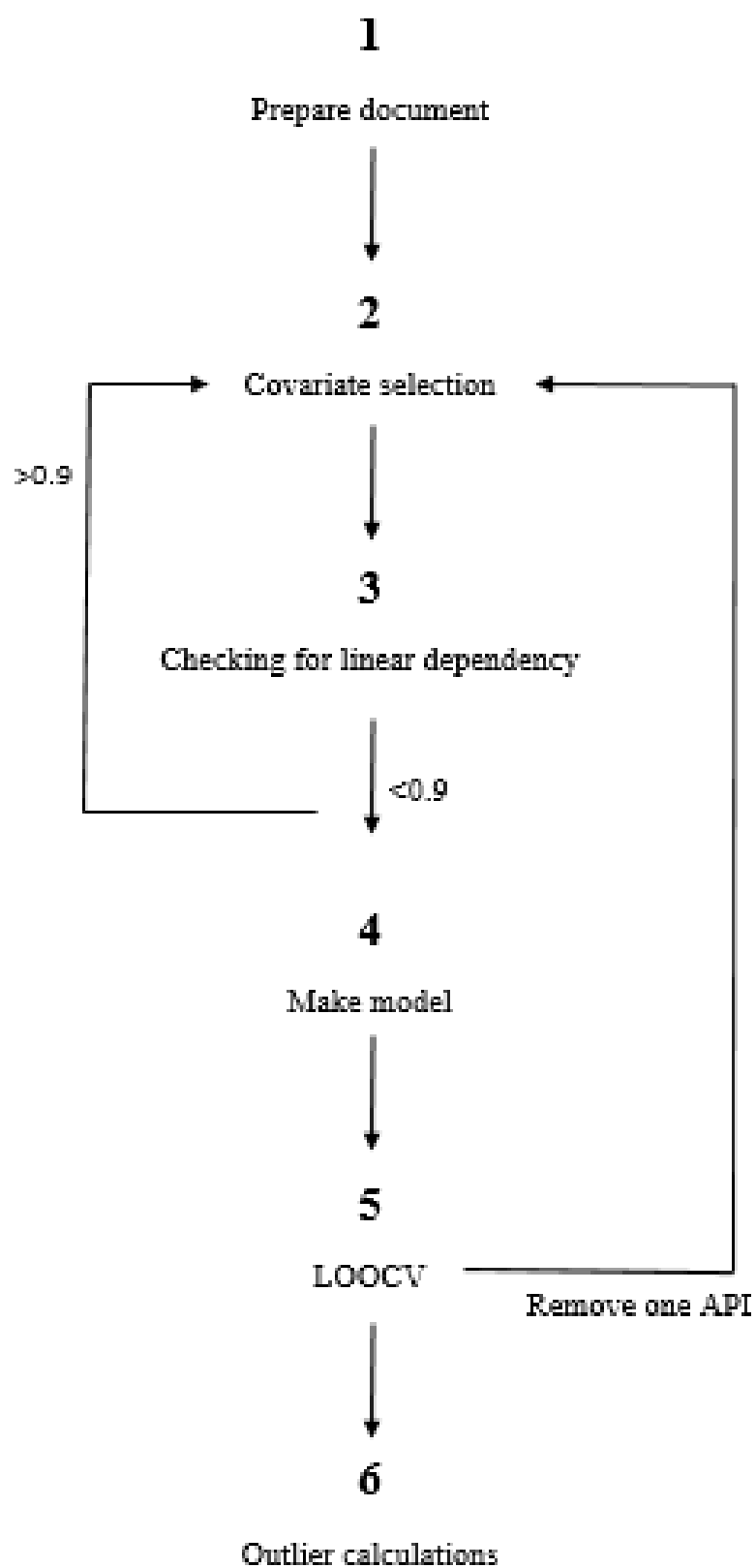
Figure 40 Formulation 6Ps at 1 month , measured with XRPD (top), PLM (middle) and DSC (bottom)

6.2. MLR coding and additional data

6.2.1. R codes for covariate selection and model building

```
library(xlsx)
library(leaps)
setwd("C:/Users/paxgf/Documents/R")
PS<-read.xlsx("all_v4.xlsx",sheetIndex=4)
sub<-regsubsets(logStab~MW+LogP+b.pKa+Atom.count+Av.Pol+TPSA+
  Bond.count+Chiral.at+Hbond.acc+Hbond.don+
  Ring.count+Rot.bond+Alip.at.count+Aro.At.count+
  ASA+HLB+logS0+Mol.Pol+PSA+VDwaal.SA+Mp.Chemspider+
  ali.ring.count+Aro.ring.count+Ccount+Fcount+Ncount
  +Ocount+Scount+ClCount+heteroatom+hetero.carbon+
  sp3+sp2+Tg.SD+GT.Tg,data=PS,nbest=1,nvmax=3,
  force.in=NULL,force.out=NULL,method="exhaustive")
sum.out<-summary(sub)
summary(sub)$adjr2
as.data.frame(sum.out$outmat)
plot(sub, scale = "adjr2", main = "Adjusted R^2 PVPVA SD")
attach(PS)
summary(lm(logStab~GT.Tg+Ncount+Ar.At.count))
cor(Ncount,GT.Tg)
```

A step by step overview of the modelling process for clarification and transparency.



- 1) Combining in one document all physicochemical properties, T_g and stability of formulations for one combination of polymer and manufacturing method. Import the document into R.
- 2) Covariate selection using the leaps package. Include all physicochemical properties and T_g and test against the measured stability. Set maximum number of output covariates to three and use “exhaustive” search method. This will include backwards, forwards and sequential. This step will select which covariates are most highly correlated with the stability by testing all possible combinations of one, two and three covariates. The results from the covariate selection are shown in a table and can be depicted in a graph.
- 3) Once the three most relevant covariates have been selected, each pair of covariates are checked for linear dependency using the cor function. If a covariate is found to have higher correlation than 0.9 with another covariate the covariate selection is repeated (step 2) but without that property. This might give three completely new covariates or just exchange the one left out for a new one. These new covariates are then checked for correlation and these two steps repeated until three covariates with no linear dependence have been found.
- 4) The three uncorrelated covariates are then used to make an MLR using the lm function. This is done using the data from all ten formulations for the combination of polymer and manufacturing method. The model is checked for R^2_{adj} and statistical significance of each covariate ($p < 0.05$). The R^2_{adj} is compared to a two-covariate model with the two most significant covariates for the three-covariate model. A substantial increase in R^2_{adj} would be expected by adding a third covariate.
- 5) Once the model has been made a leave-one-out cross validation (LOOCV) was performed on the model. This was done by removing one API at a time and repeating steps 1-4. Thus, one model was made for each set of nine formulations. That model was then used to predict the stability of the API that was left out and the mean square error (MSE) calculated between the predicted and measured stability.
- 6) A further step to check for outliers in predicted values, that is where squared error between measured and predicted stability was much higher for one API than all the others. First the median, first (Q1) and third (Q3) quartile were determined. The interquartile range (IQR) was calculated by subtracting Q1 from Q3. The lower limit for outliers was then determined as the 1.5 times the IQR subtracted from Q1. The upper limit similarly was calculated as 1.5 times the IQR added to Q3. If any squared errors calculated in step 5 were above or below these limits they were classed as outliers. An outlier value would then be skipped when calculating the MSE

6.2.2. Confidence interval – R codes

```

t10<-2.22814
t20<-2.11991
t30<-2.05553
sigma<-3.481071228

> X<-matrix(c(1, 97.2, 173.00, 1,
+           1, 97.2, 145.00, 1,
+           1, 98.5, 166.00, 0,
+           1, 95.4, 125.00, 2,
+           1, 97.6, 121.00, 4,
+           1, 90.8, 81.00, 0,
+           1, 98.5, 158.00, 1,
+           1, 101.2, 152.00, 2,
+           1, 88.2, 44.00, 1,
+           1, 96.3, 255.00, 2),ncol=4, byrow = TRUE)

x10<-X[10,]
rot10<-sigma^2*t(x10)%*%solve(t(X)%*%X)%*%x10
rot10*t20

```

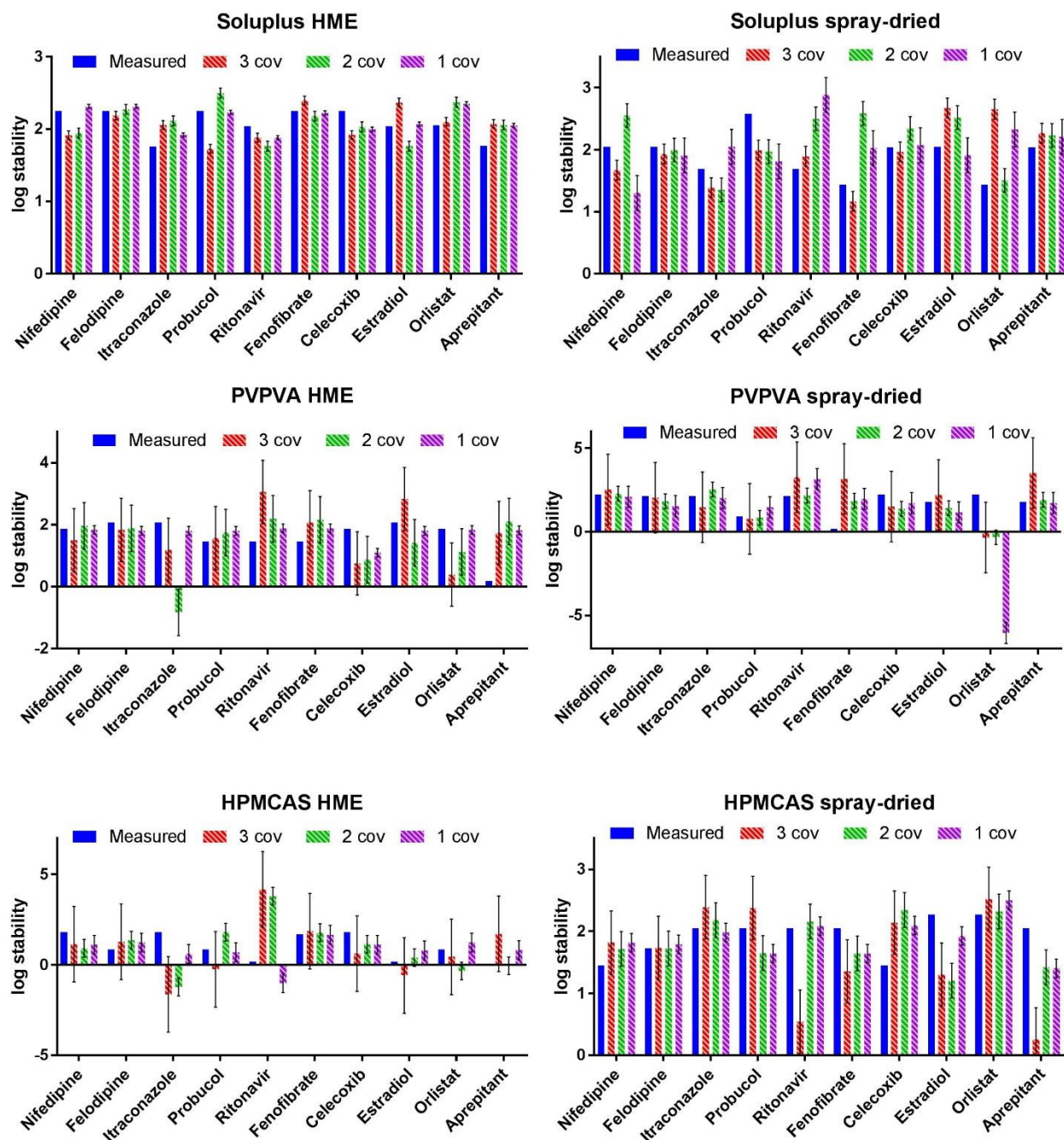


Figure 41 shows the LOOCV for each model. The measured stability and the predicted stability using three, two and one covariate models and the corresponding MSE.

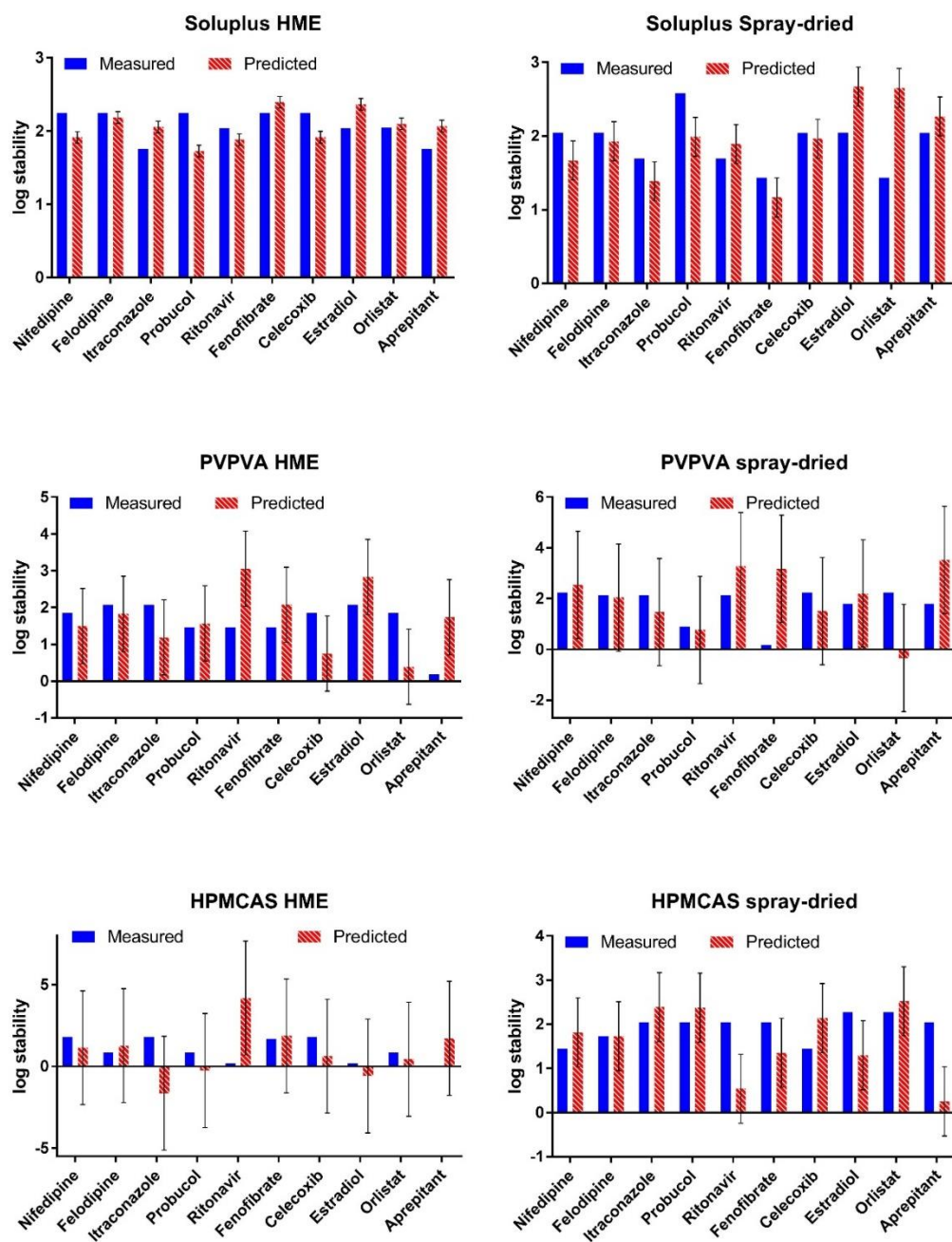


Figure 42 Shows the LOOCV for each of the six models using the MSE of all errors, including the outlier values.

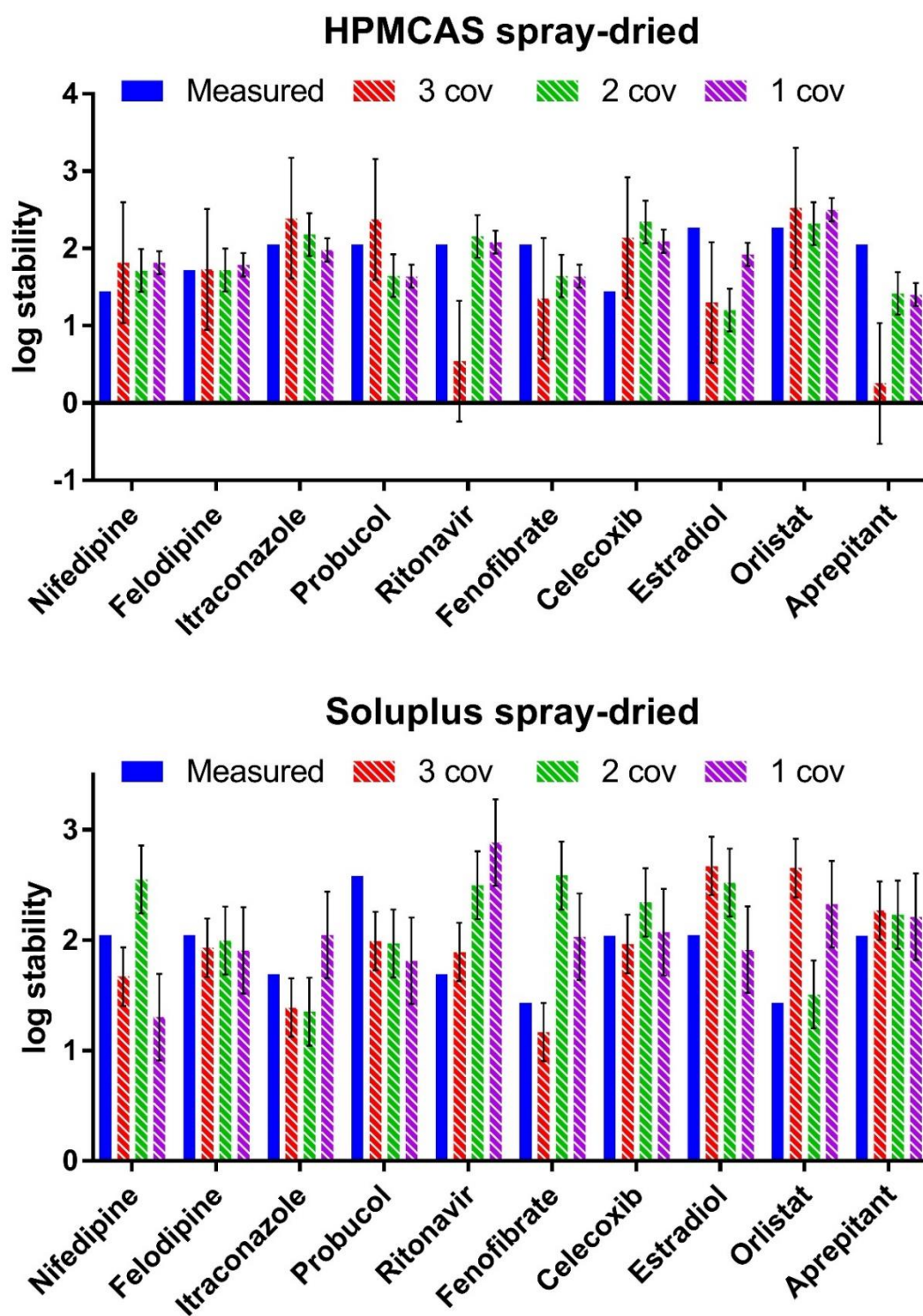


Figure 43 LOOCV for SH and HS, one, two and three covariate models using MSE of all errors.

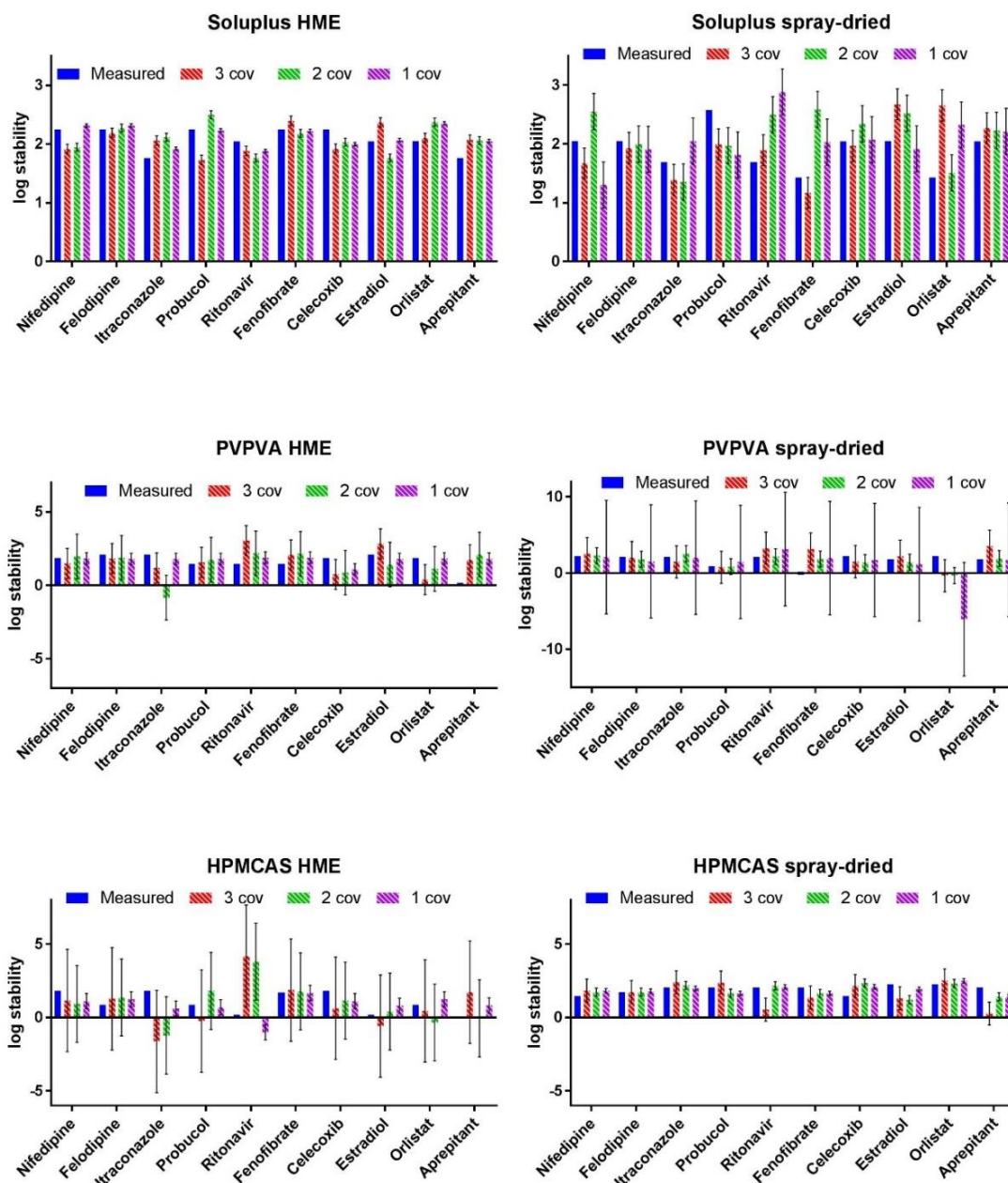


Figure 44 LOOCV for all models; one, two and three covariate models. Error bars represent MSE calculated with all errors.

Table 18 LOOCV for SH. First row shows overall model built on all data. Second row (SH w/nF) shows the best model for all data which includes nF. This model was not used due to the low number of flourine atoms in the test APIs. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. Abbreviations used: int – intercept, k – constant of covariate.

	Int	k	TPSA	k	NChir	k	HBA	R ² _{adj}	Meas	Pred	Error ²
SH	2.3072027	0.003007	TPSA	-0.0546909	nChir	-0.082152	HBA	0.89			
SH w/nF	2.6754941	-0.040059	nF	-0.1317876	nAliphRing	-0.000905	MW	0.92			
Nifedipine	2.321430	0.02443	Sp ²	-0.001020	Mp	-0.104868	5	0.91	2.25	1.91	0.11
Felodipine	2.655741	-0.03885	nF	-0.000879	MW	-0.131459	nAliphRing	0.92	2.25	2.19	0.00
Itraconazole	0.913254	-0.06552	Mp	0.025467	nChir	-0.002489	T _{g,pred}	0.94	1.76	2.06	0.09
Probucol	2.749834	0.00422	40.46	-0.002610	Mp	-0.010342	nBond	0.95	2.25	1.73	0.27
Ritonavir	2.437779	-0.02323	nF	-0.060297	nChir	-0.052483	6	0.92	2.04	1.88	0.03
Fenofibrate	2.76E+00	-4.36E-02	nF	-1.50E-01	nAliphRing	-1.01E-03	MW	0.98	2.25	2.39	0.02
Celecoxib	2.429843	-0.04504	b.pK _a	0.053757	HBD	-0.007728	T _{g,meas}	0.96	2.25	1.92	0.11
Estradiol	2.74E+00	-3.44E-02	nF	1.28E-01	nS	-1.37E-03	MW	0.98	2.04	2.37	0.11
Orlistat	2.677574	-0.04106	nF	-0.131272	nAliphRing	-0.000898	MW	0.92	2.05	2.10	0.00
Aprepitant	2.508016	-0.01173	nAr	-0.131891	nAliphRing	-0.013333	nRot	0.98	1.76	2.07	0.09
										MSE	0.08

Table 19 LOOCV models, R^2_{adj} and error for SS. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. The orlistat model gives much larger error than the rest. Orlistat is poorly predicted as it has the lowest T_g and fewer number of aromatic rings than the rest of the APIs or none compared to 1-5. Abbreviations used: int – intercept, k – constant of covariate.

	int	k	Mol.Pol	k	nN	k	$T_{g,meas}$	R^2_{adj}	Meas	Pred	Error ²
SS	-3.8637	0.01512		-0.163		0.08081		0.89			
Nifedipine	-0.3768	-0.0082	TPSA	0.23089	nS	0.04112	71.2	0.90	2.05	1.67	0.14
Felodipine	-3.3164	0.07286	ClogP	-0.099	1	0.07509	67.8	0.90	2.05	1.93	0.01
Itraconazole	-4.1345	0.0155	71	-0.1928	8	0.08522	70.0	0.94	1.69	1.39	0.09
Probucol	-1.8606	-0.0164	nF	-0.0604	0	0.05745	67.0	0.97	2.58	1.99	0.35
Ritonavir	-3.9781	0.01715	75	-0.1607	6	0.08127	68.3		1.69	1.89	0.04
Fenofibrate	2.01778	-0.1377	nRing	-0.0823	nRot	0.00162	vdW-SA	0.90	1.43	1.17	0.35
Celecoxib	-3.8615	0.01577	36	-0.1655	3	0.08029	71.7	0.89	2.04	1.96	0.01
Estradiol	-3.7694	-1.8758	nHet/C	-0.084	0	0.09779	68.0	0.97	2.05	2.67	0.39
Orlistat	-1.918	-0.124	logS0	-0.2705	nArRing	0.05483	58.5	0.97	1.43	2.65	1.49
Aprepitant	-3.7032	0.07092	ClogP	-0.1028	4	0.08163	74.2	0.94	2.04	2.27	0.05
										MSE	0.29

Table 20 LOOCV models, R^2_{adj} and error for KH. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. Three models give much larger error than the rest, the ritonavir, orlistat and aprepitant. For ritonavir the poor prediction of stability is mostly down to PSA being chosen as a covariate. Ritonavir has much higher PSA than all the other APIs or 145 compared to 40-100. Orlistat is poorly predicted due its low T_g . Oppositely, the poor prediction of aprepitant stability is because of the high melting point and T_g which is extreme compared to the other APIs. Abbreviations used: int – intercept, k – constant of covariate.

	Int	k	$T_{g,pred}$	k	mp	k	HBD	R^2_{adj}	Meas	Pred	Error ²
KH	-11.211	0.153614		-0.0112		-0.2355		0.81			
Nifedipine	-11.178	0.1533	97.2	-0.0116	173.00	-0.2211	1	0.86	1.85	1.50	0.13
Felodipine	-10.858	0.14934	97.2	-0.011	145.00	-0.2246	1	0.82	2.07	1.83	0.06
Itraconazole	-3.8335	0.14165	logS0	-0.0187	166.00	0.09077	$T_{g,meas}$	0.99	2.07	1.19	0.77
Probucol	-4.1218	0.09274	$T_{g,meas}$	-0.0185	125.00	0.12805	logS0	0.96	1.46	1.57	0.01
Ritonavir	-13.38	0.17025	97.6	-0.0142	121.00	0.01057	PSA	0.95	1.46	3.05	2.53
Fenofibrate	-9.5754	0.1387	90.8	-0.0117	81.00	-0.2868	0	0.93	1.46	2.07	0.37
Celecoxib	1.4472	-0.2572	nF	0.22951	nAliphRing	0.01873	HLB	0.95	1.85	0.75	1.21
Estradiol	-16.093	0.20934	101.2	-0.0149	152.00	-0.3971	nS	0.85	2.07	2.83	0.58
Orlistat	-6.4222	0.0896	88.2	-0.2238	nF	-0.0419	sp3	0.89	1.85	0.39	2.13
Aprepitant	-2.244	0.04864	$T_{g,meas}$	-0.0046	255.00	-0.2674	nS	0.88	0.18	1.73	2.42
										MSE	1.02

Table 21 LOOCV models, R^2_{adj} and error for KS. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. Two models give much larger error than the rest, the fenofibrate and orlistat. Fenofibrate has low values for all the selected covariates, especially the T_g which is the second lowest and considerably lower than the rest. Orlistat has an extremely low value for the predicted T_g . Abbreviations used: int – intercept, k – constant of covariate.

	Int	k	nAr	k	nN	k	$T_{g,pred}$	R^2_{adj}	Meas	Pred	Error ²
KS	-5.2524	-0.1185		0.3897		0.07854		0.80			
Nifedipine	-5.7585	-0.1282	6	0.40965	2	0.08488	97.2	0.80	2.23	2.54	0.10
Felodipine	-5.1091	-0.117	6	0.38843	1	0.07679	97.2	0.78	2.13	2.04	0.01
Itraconazole	-20.186	0.19745	nRot	-0.1132	nC	0.23803	98.5	0.86	2.13	1.47	0.43
Probucol	-5.3202	-0.1228	12	0.40547	0	0.07926	95.4	0.74	0.90	0.77	0.02
Ritonavir	-27.329	0.2297	nRot	-0.0895	nC	0.30515	97.6	0.98	2.13	3.28	1.32
Fenofibrate	11.913	0.20065	HBA	-0.0655	sp ³	-0.0975	$T_{g,meas}$	0.92	0.18	3.17	8.97
Celecoxib	-4.2632	-0.1283	17	0.4147	3	0.06813	98.5	0.94	2.23	1.51	0.52
Estradiol	-7.3156	-0.1228	6	0.37552	0	0.10137	101.2	0.81	1.80	2.21	0.17
Orlistat	-18.102	-0.1082	nF	3.16905	nHet/C	0.19395	88.2	0.95	2.23	-0.34	6.62
Aprepitant	-8.628	0.29581	nHet	-0.1819	sp ²	0.10962	96.3	0.95	1.80	3.52	2.97
										MSE	2.11

Table 22 LOOCV models, R^2_{adj} and error for HH. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. Two models give considerably larger error than the rest, the itraconazole and ritonavir models. Itraconazole has by far the largest number of rings in its structure or 7 compared with 1-4 in other compounds. Ritonavir has the highest TPSA of all the compounds or 145 compared to 64-107 for the other APIs. Abbreviations used: int – intercept, k – constant of covariate.

	Int	k	ClogP	k	HBD	k	nS	R^2_{adj}	Meas	Pred	Error ²
HH	2.4724	-0.1243		-0.8485		0.77114		0.83			
Nifedipine	1.2813	0.00601	107.77	-0.7801	1	0.54212	0	0.91	1.81	1.15	0.43
Felodipine	2.6038	-0.1374	3.44	-0.8593	1	0.76556	0	0.86	0.85	1.27	0.18
Itraconazole	3.4154	0.01124	TPSA	-0.6324	nRing	-0.0839	nAliph	0.90	1.81	-1.64	11.87
Probucol	1.2017	0.01704	TPSA	-0.6193	2	-0.0392	nAliph	0.85	0.85	-0.25	1.20
Ritonavir	10.942	0.02514	TPSA	0.02811	nAr	-0.1149	$T_{g,meas}$	0.90	0.18	4.18	16.02
Fenofibrate	2.5357	-0.1266	5.28	-0.8766	0	0.78583	0	0.80	1.68	1.87	0.03
Celecoxib	0.8306	0.21391	nO	-0.6634	1	0.46074	1	0.84	1.81	0.63	1.39
Estradiol	1.0040	0.01348	TPSA	-0.4808	2	-0.2351	nChir	0.83	0.18	-0.59	0.58
Orlistat	2.6196	-0.1588	8.11	-0.8904	1	0.8864	0	0.85	0.85	0.44	0.16
Aprepitant	2.4105	0.13217	b.pK _a	-0.3975	nChir	-0.4936	0	0.89	0.00	1.71	2.93
										MSE	3.48

Table 23 LOOCV models, R^2_{adj} and error for KS. At the top are the covariates chosen in the overall model. Where the LOOCV process has chosen other covariates these are shown in the table instead of the covariate value. First row shows overall model built on all data. The following rows show the best model for each LOOCV, when each API is left out. In the nifedipine row, nifedipine was left out and its properties used to predict the stability. The squared error (Error) was calculated between the predicted (Pred) and measured (Meas) error and at the bottom the mean square error (MSE) is shown. Two models give much larger error than the rest, the ritonavir and aprepitant. Ritonavir has the highest value for ASA of all the APIs. Aprepitant is one of only two APIs that contains fluorine atoms and has seven of them while celecoxib, the other F containing API only has three. Abbreviations used: int – intercept, k – constant of covariate.

	int	k	nRing	k	nRot	k	nN	R^2_{adj}	Meas	Pred	Error
HS	0.95282	0.33131		0.05485		-0.2155		0.87			
Nifedipine	2.59309	0.05206	b,pK _a	0.04448	HLB	-2.4242	nHet/C	0.84	1.45	1.82	0.14
Felodipine	0.95555	0.3307	2	0.05475	6	-0.2152	1	0.85	1.72	1.73	0.00
Itraconazole	0.79029	0.38676	7	0.0585	11	-0.2188	8	0.93	2.05	2.39	0.12
Probucol	2.58912	0.09952	nF	0.05854	nHet	-3.4698	nHet/C	0.92	2.05	2.38	0.11
Ritonavir	1.98783	0.24914	ClogP	-0.0018	ASA	-0.4962	nS	0.91	2.05	0.54	2.27
Fenofibrate	1.61262	0.00739	nAtom	-0.0049	TPSA	0.10695	nChir	0.97	2.05	1.35	0.48
Celecoxib	2.4356	0.0648	b,pK _a	-0.0229	HLB	-0.07	nN	0.95	1.45	2.14	0.48
Estradiol	7.48011	0.00189	MW	-0.0031	TPSA	-0.0555	$T_{g,pred}$	0.93	2.27	1.30	0.94
Orlistat	0.88781	0.34301	1	0.0664	23	-0.2359	1	0.87	2.27	2.52	0.06
Aprepitant	2.18665	0.03377	nChir	-0.046	HLB	-0.1852	nF	0.96	2.05	0.26	3.22
										MSE	0.78

6.3. Published work



Review

Support Tools in Formulation Development for Poorly Soluble Drugs

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ABSTRACT

The need for solubility enhancement through formulation is a well-known but still problematic issue because of the numbers of poorly water-soluble drugs in development. There are several possible routes that can be taken to increase the bioavailability of drugs intended for immediate-release oral formulation. The best formulation strategy for any given drug will depend on numerous factors, including required dose, shelf life, manufacturability, and the properties of the active pharmaceutical ingredient (API). Choosing an optimal formulation and manufacturing route for a new API is therefore not a straightforward process. Currently, there are several approaches that are used in the pharmaceutical industry to select the best formulation strategy. These differ in complexity and efficiency, but most try to predict which route will best suit the API based on selected molecular parameters such as molecular weight, lipophilicity (logP), and solubility. These methods range from using no tools, trial and error methods through a variety of complex tools from small *in vitro* or *in vivo* experiments or high throughput screening, guidance maps, and decision trees to the most complex methods based on computational modelling tools. This review aims to list available support tools and explain how they are used.

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Introduction

Oral drug delivery, specifically solid oral dosage forms, for example, tablets and capsules, is the preferred administration route for the majority of medicines. Such formulations are generally stable, easy to produce, and have accurate doses, in addition to being simple to administer and having generally good compliance. Therefore, most drugs on the market and in development are solid formulations for oral administration. For these to give reproducible and effective *in vivo* plasma concentrations, the drugs need to be readily released and absorbed. However, many new chemical entities (NCE) have a narrow absorption window, being absorbed almost exclusively in the upper part of the small intestine. It is therefore of great importance that such drugs are released completely in the gastrointestinal tract before absorption to avoid low bioavailability.¹ Unfortunately, according to a recent study by Di et al.,^{2,3} up to 75% of new candidates in drug development have low solubility. At the same time, around 40% of marketed drugs are poorly soluble.⁴ It is therefore clear that poor solubility continues to be a major obstacle in the development of new medicines.⁵

There are a number of methods that can be used to improve the solubility of a drug through the formulation route. Formulation

strategies or enabling formulations include particle size reduction, lipid-based vehicles, use of cyclodextrin complexation, formation of salts, polymorphs, and cocrystals, and solid dispersions (as reviewed by Williams et al.⁶ and Singh et al.⁷). Identifying which approach gives sufficient solubility and satisfactory chemical and physical stability can take substantial resources and time, especially if many approaches have to be tested to find a suitable one. Furthermore, for those NCEs that are not compatible or soluble in the most commonly used excipients, there is a large number of extra tests that need to be performed to find suitable excipients, ratios of excipients, and processing conditions. Thus, formulation development can potentially be labor intensive and cost and time consuming in addition to requiring a large amount of active pharmaceutical ingredient (API). This latter point is an important issue as, especially in the early development phase, there is sometimes a limited amount of compound available, which can restrict the number and type of formulation approaches that it is possible to test. Without a suitable formulation, an NCE will not be able to progress in the development process.^{8,9} Poorly water-soluble drugs intended for oral delivery hence carry considerable risk which can cause higher cost, difficulties in preclinical and clinical trials due to reduced and inconsistent exposure, and therefore increase time to market. There is thus a need for improved efficiency, precision, and prediction in drug development.

As poor solubility is often known early in the development of an NCE, it is important to be able quickly to identify which formulation strategies are available in each specific case. Instead of dealing with

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solubility issues on a project basis, relying to some degree on trial and error, a support tool would be very valuable for giving indications of possible solutions early without having to conduct many experiments. As noted, quantities of NCEs are usually limited early in development and such a tool could greatly optimize the use of what is available. There are some tools currently available that can be beneficial in the earlier or later stages of drug development.^{10,11}

The optimal formulation for each specific drug depends on numerous factors, including the required dose, administration route, shelf life, manufacturability, and the physicochemical properties of the drug. Choosing the optimal formulation for a drug is therefore not a straightforward process, and a poor choice of formulation can have devastating effects on the development process for a drug, such as leading to poor clinical data, which in turn can necessitate drug reformulation and prolonged clinical trials, or even termination of the project.¹² Currently, there are several ways that can be used to select the best formulation route for a new drug candidate. These differ in complexity and efficiency and range from trial and error formulations, small scale *in vitro* or *in vivo* experiments, guidance maps, and decision trees to complex computational modelling tools. Figure 1 shows how and where in the formulation development process such support tools can be used.¹³ This review aims to shine some light on the available support tools in formulation development and how they can be used.

High Throughput Screening

In any formulation development process, there is a set of most commonly used excipients which are first tested with an NCE. If those are unsuitable, there is a long list of additional excipients, ratios, and processing conditions that can be tested. If no suitable formulation with adequate solubility is found, the drug candidate cannot advance in the development cycle. In such cases, high throughput screening (HTS) is extremely valuable, where it is possible to test a large number of combinations of excipients or formulations using only a very small quantity of API. Furthermore, it is less labor and time intensive than performing the same experiments on a larger scale.^{8,9}

For example, Tandem Nano (www.tandemnano.com) has developed a novel HTS method for aiding the formulation development

process for nanoparticle formulations. The first part of this emulsion-template freeze-drying screen-based process is building a library of freeze-dried formulations, using combinations of accepted excipients. The second stage includes characterization of the formulations, such as particle size and the capability of redispersion, revealing which excipient combinations work best for the selected API. Using this screening method as many as 1000 freeze-dried particle formulations can be made using as little as 1 mg of API each, thus allowing a rapid selection of a viable formulation without wasting much material.¹⁴ In a recent study, McDonald et al.¹⁵ used this novel HTS for the first time to make a nanoparticle formulation with efavirenz, a non-nucleoside reverse transcriptase inhibitor. Water-soluble polymers and surfactants, 7 of each, were chosen for the study, making 49 formulations. These were freeze dried and characterized to determine which polymer and surfactant best stabilized the efavirenz nanoparticles. Only one polymer, polyvinyl alcohol, was capable of making nanoparticles that completely redispersed to solid drug nanoparticles. The study showed that the screening method was able successfully to optimize the nanoparticle formulation for efavirenz. This emulsion-template freeze-drying platform potentially has broad application for formulation optimization of poorly soluble or poorly bioavailable APIs.¹⁵

A microscreening method developed by ALZA Corporation, now owned by Johnson & Johnson, rapidly produces and tests hundreds of formulations on a small scale, using only tens of micrograms of API. In this method, drug and excipients are dissolved in an appropriate solvent, such as n-propanol or acetone, and the solution is dispensed into the wells of a 96-well plate. The solvent is then removed using a vacuum centrifuge evaporator leaving the formulation remaining at the bottom of each well, containing 10–40 µg of drug and around 0.4 mg of excipients. The solubility of each formulation can then be tested by adding an aqueous medium into the wells, incubating at 37°C and measuring the drug concentration in solution using high-performance liquid chromatography or ultraviolet spectroscopy.⁹

A screening method for nanoprecipitate formulations, similar to the one described previously, was developed by McDonald et al.¹⁶ The drug triclosan and a polymer, each in varying concentrations with and without the addition of a surfactant (1 of 3), was mixed in a 96-well plate with an automatic liquid-handling robot. The solution was analyzed using dynamic light scattering to check for nanoprecipitation. The samples were then freeze-dried. After redispersion in water, the formulations were reexamined with dynamic light scattering for precipitation. In addition, the formulations were subjected to zeta-potential analysis, and drug concentration in solution was measured by ultraviolet spectroscopy. Size distribution and morphology were examined using scanning electron microscopy. In this study, 252 formulations containing triclosan were produced and tested, many of which showed improved functional properties, in some cases more than 10-fold increase in antimicrobial activity when IC₅₀ was tested in *Escherichia coli* and compared to triclosan in aqueous solution.¹⁶

Dai et al.⁸ used the microscreening method to identify a suitable solid dispersion formulation for a compound, using <10 mg. Each formulation consisted of the compound, a polymer, and a precipitation inhibitor. Nine enteric polymers and 7 precipitation inhibitors and their combinations were studied. Each formulation was produced with an evaporation method, and the film produced was then dissolved in simulated intestinal fluid to determine solubility.⁸

The HTS methods described previously can be divided into 2 groups as provided in Table 1. The solvent casting method requires less material as the samples are dispensed as a liquid into a 96-well plate. However, the limitation is that both the API and potential excipients intended for screening have to be soluble in the same

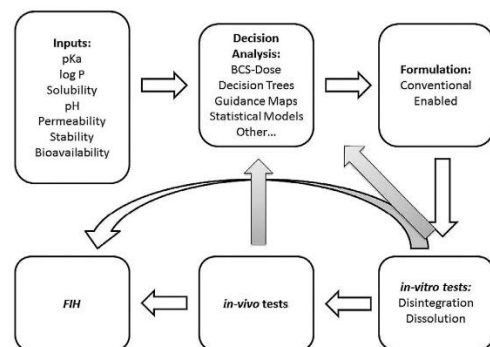


Figure 1. A schematic summary of the formulation decision process in many pharmaceutical companies. The decision analysis step is where the process can be expedited with the use of relevant support tool. Depending on the outcomes of *in vitro* and *in vivo* experiments carried out, the formulation development can go back to the decision analysis step. This process can be repeated until a suitable formulation is found. FIH, first-in-human or the first time the formulation is tested in humans. Adapted from Pearce.¹³

volatile organic solvent. In addition, after solvent evaporation, the drug can change its solid form, taking an amorphous form or a different crystal polymorph form. However, as a fast screening method in early development, it can give valuable information fast. Freeze drying might be a more relevant production method with respect to final formulation production. It requires higher concentrations of API per formulation test compared to the solvent casting method but can in turn give more detailed information. However, most tests used to characterize the produced formulations were not carried out as HTS in the well plate but were done manually for each test, where a fraction of the formulation was transferred to a cuvette, vial or other appropriate test holder. The only true HTS characterization done on the formulations in the well was a visual observation to check for precipitation. Therefore, although the freeze-dried HTS can give more detailed information about the formulations produced, solvent casting is a truer HTS where both production and characterization is automated and performed in the well plate.

Classification Systems

The Biopharmaceutics Classification System (BCS) is a basic tool used in drug development. The graphic design in Table 2 gives a brief indication whether an API will have bioavailability problems and if this is likely to be due to limited solubility or permeability. The BCS classes drugs into 4 categories based on solubility and permeability. Class I are drugs which are both highly permeable and soluble, class II has high permeability but low solubility, whereas class III is opposite with high solubility and low permeability. Class IV contains drugs which are both poorly permeable and soluble. APIs which are absorbed $\geq 90\%$ in humans are classed as highly permeable. The criteria for high solubility are that the highest dose strength of the API is soluble in 250 mL of aqueous solution over the pH range 1–7.5.^{17–19} This information is useful in formulation development (Table 2), and attempts have been made to increase the value of this tool by adding further parameters to the system as described in the later discussion.^{20–23}

A revised classification system based on BCS was devised by Butler and Dressman¹⁹ to make it more useful in formulation development. It was proposed that in the standard format, it is too conservative in the assessment of absorption-limiting solubility and dissolution rate. Therefore, the new modified system (Fig. 2), called the Developability Classification System increases the volume for assessing solubility of a dosage form to 500 mL from 250 mL and uses a fasted-state simulated intestinal fluid as the medium. It also divides class II into 2 subgroups, IIa and IIb, where 2a is dissolution rate limited and 2b is solubility limited. The boundary between the 2 subclasses is determined by the solubility-limited absorbable dose, which is calculated from an estimate of small intestine solubility, fluid volume, and a permeability-dependent multiplier.¹⁹

Leane et al.²⁴ have been working on developing a Manufacturing Classification System, a supportive tool to help rational selection of an optimal processing route for a specific API. The system is, similar to the BCS, based on 4 classes with increasing risk associated with each class. The classes are (I) direct compression, (II) dry granulation, (III) wet granulation, and (IV) other technologies, which includes melt

granulation and liquid dispensing technology. This tool is not fully developed yet but is promising as an aid in formulation development.²⁴

Guidance Maps

Guidance maps are developed to give indications about the probability of an event. They are a visual aid to show the probability of a method being successful. For instance, a guidance map could address the probability of a solubilization technique to give sufficient absorption for a drug to be efficient and to give indication of which technique is most suitable for a drug or even how much drug loading can be used for a drug. Some guidance maps are used to predict the solubilization effect of a certain technique for a single drug. In such cases, each technique could have a separate map. These can for example be based on logP and dose to solubility ratio, as in the examples of the first 2 guidance maps in Figure 3. The 2 guidance maps in panels c and d show overall technology guidance maps which give an indication on which formulation route to choose for an API based on melting point (T_m), logP, and dose. There are some challenges making these guidance maps. Because they are based on solubility and not permeability, they do not always give a correct indication of bioavailability. In some cases, one salt or polymorph can have the same or lower solubility as another one but have higher bioavailability. This can happen if one salt form, although being more soluble than another one, does not form permeable ion pairs (private communication, R. Shanker).

Friesen et al.²⁵ constructed a guidance map based on 2 physicochemical properties of >130 drug candidates that had been formulated as solid dispersions in hydroxypropyl methylcellulose acetate succinate (HPMCAS). The 2 properties they plotted against were lipophilicity (logP) and T_m/T_g ratio. The T_m/T_g ratio has been linked with a drug's susceptibility to crystallize. Drugs with high T_m have a strong crystallization tendency, whereas a low glass transition temperature (T_g) indicates a low kinetic barrier for crystallization to start. Thus, a high T_m/T_g ratio indicates that a drug has a high crystallization tendency and a low barrier for crystallization, meaning an unstable amorphous form. Using a polymer with a high T_g in the solid dispersion, such as HPMCAS, can stabilize the mixture by increasing the mixture's T_g compared to the drugs on their own. However, with increasing drug loading, the stabilizing effect of the carrier polymer decreases. Therefore, the drug-loading capability of a solid dispersion decreases with an increased T_m/T_g ratio. High lipophilicity is associated with poor solubility which also limits the drug loading of a solid dispersion. Taking into account these limiting factors for drug loading of solid dispersions, stability, and dissolution, the authors divided drugs into 4 groups based on their position on the map. These 4 groups indicate the maximum drug loading that can be stabilized with HPMCAS as shown in the guidance map in Figure 4. Group 1 in panel i contains the most stable and soluble drugs. Drugs in this group can generally be successfully formulated in solid dispersion with high drug loading, for example, >50% by weight, while still upholding acceptable physical stability and dissolution rate. When moving upward in the map, the physical stability and drug loading decrease. To maintain the stability and dissolution rate, the drug loading can only be 35–50% or 10–35% for groups 2 and 3, respectively. Group 4 has increased lipophilicity making the dissolution rate the limiting factor for drug loading, which is the same as for group 3, 10–35%.²⁵ This guidance map can also be used to indicate where other considerations have to be taken with a formulation, for example, are the drugs good glass formers? For drugs in group 3, the packaging and storage conditions are very important because of limited physical stability. Drugs in group 4 in contrast, because of dissolution limitations, can be sensitive to changes in particle size due to particle size-dependent dissolution.^{6,26}

Table 1
The Main Features of the 2 Groups of HTS Methods Discussed

Production Method	Amount Required	Attributes Tested
Freeze drying	1 mg	Redispersibility, size distribution, z potential, concentration, morphology
Solvent casting	10–40 μ g	Solubility

Table 2
Formulation Strategies Based on BCS Class

Solubility	
Permeability	Class I Simple capsule or tablet
	Class II Particle size reduction Micronization, nanocrystals Crystal modification Salt formation, polymorphs, cocrystals, amorphization Surfactant Nanoparticle technology Solid dispersion Lipid formulations Liquid-filled capsules, self-emulsifying systems
	Class III Simple capsule or tablet Absorption enhancers
	Class IV Combination of BCS 2 and absorption enhancers

Adapted From Ku²⁰ and Kawabata et al.²¹

In Vivo Studies

There are several factors that can limit the bioavailability of a drug, for example, dissolution rate, solubility, and permeability. Which formulation strategy increases bioavailability the most depends on which factor is the limiting one. This is why an early *in vivo* study can be beneficial as a tool to determine which limiting factors have to be addressed in a formulation development. Such a study can be performed in rats or possibly dogs where the exposure of a drug from a suspension and a solution formulation is compared. As these 2 simple formulations are being compared to gather information about limitations in bioavailability, no extrapolation of exposure to humans will be done. Such a study is intended to test neither the bioavailability in humans nor the best formulation candidates, just as an early indicator of what kind of

formulation route will be beneficial to the API. Whether there is a difference between the 2 formulations and how the exposure differs indicate what the limiting factor is and what formulation strategy can be used to improve the bioavailability (Fig. 5). Thus, if the 2 formulations give equivalent exposure and have high bioavailability, this indicates the drug is BCS class I. Similarly, if the formulations are equivalent but have low bioavailability, the API may be considered a BCS class III drug, limited by permeation or possibly first-pass metabolism. For drugs from these 2 categories, conventional formulation strategies can be used in most cases. If the exposure from solution is much higher than that from suspension, this indicates that the bioavailability of the solid API is limited by solubility or dissolution rate. However, for the opposite case, where suspension gives higher exposure than solution, it might indicate chemical instability in the acidic environment of the stomach or precipitation of the drug into a less-soluble form. An enabling formulation is likely required for APIs with high solution to suspension exposure ratios to increase the solubility and dissolution rate, while an enteric coating might suffice in the case of high suspension to solution exposure ratio.^{26–29}

Decision Trees

A decision tree is a support tool where each decision in the formulation selection is represented as a step in a flow chart. Thus, the whole decision tree (or flow chart) will guide one through the steps that need to be considered when choosing the optimal formulation for an API. Each tree is built with a specific task in mind, such as the development of an oral formulation for poorly bioavailable drugs,¹¹ an *in vitro* formulation,³⁰ a first-in-man formulation,^{12,31} or even depending on type and timescale of formulation process.³² These trees vary in complexity: some are quite simple with few steps and outcomes, whereas others have many steps and possible outcomes. Most are based on the properties of the API and are quite general and not developed for a specific API. An example of a step in a decision tree could be the

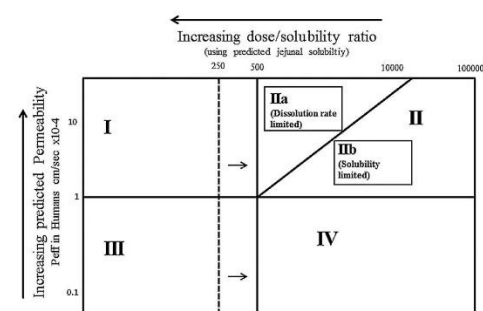


Figure 2. In the developability classification system, the dotted line shows the change in volume from the BCS. The diagonal line in class II divides it into class IIa, which is dissolution rate limited, and class IIb, which is solubility limited. Adapted from Butler and Dressman.¹⁸

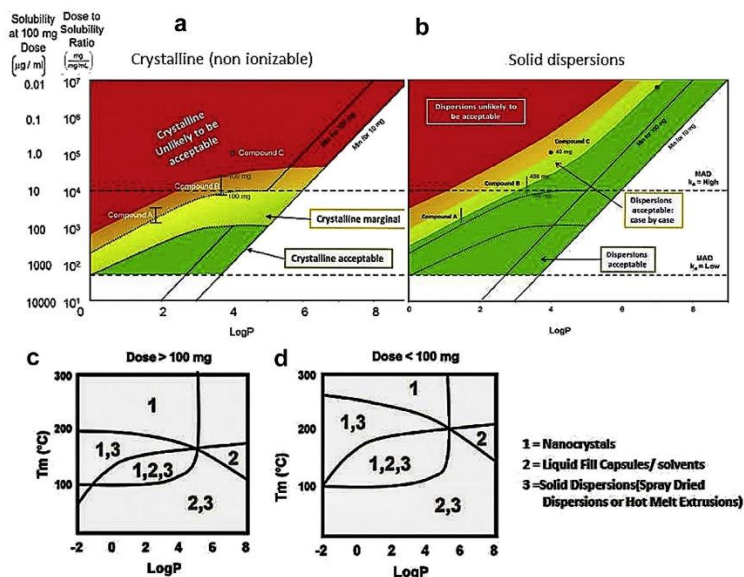


Figure 3. Two solubilization technique guidance maps are shown: (a) formulation of crystalline, nonionizable drugs and (b) solid dispersion formulation, both HME and SSD. Green indicates high probability of successful formulation and red indicates where the formulation is most likely unacceptable. The bottom 2 maps show an overall technology guidance map giving an indication of which solubilization technique to choose depending on the melting point (T_m) and lipophilicity (logP) of the API. Maps are shown (c) where the API dose is >100 mg and (d) where it is <100 mg. The formulation options are (1) nanocrystals, (2) liquid-fill capsules/solvents, and (3) solid dispersions, hot melt extruded (HME), or spray dried (SSD) (private communication, R. Shanker).

following: does the API have a T_m higher than 225°C? If the API has T_m <225°C, then it might be suitable for hot melt extrusion processing method. However, if it has a T_m of >225°C, it might be suitable for either solvent method or spray congealing, and so forth.

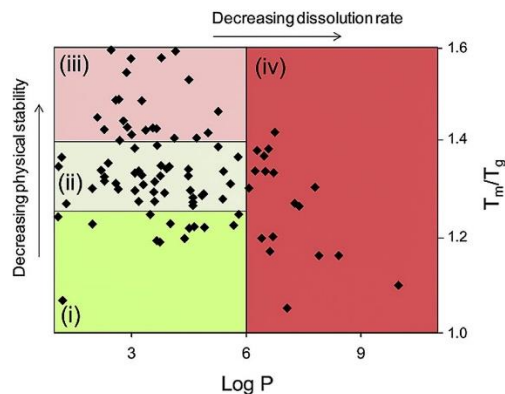


Figure 4. A guidance map indicating how much drug loading can be used, depending on which group an API belongs to, in solid dispersions with HPMCAS, while upholding an acceptable physical stability, for example, 2 years shelf life under standard storage conditions. APIs are divided into 4 groups based on their lipophilicity (logP) and the ratio between their melting point and glass transition temperature T_g . The first group can have high drug loading (>50%), the second group 35%–50% drug loading, the third and fourth groups can have 10%–35% drug loading while still having sufficient stability and dissolution rate.^{6,25}

In each case, more questions need to be answered to decide which process method is suitable for that API (Fig. 6).³³

Branchu et al.¹¹ have created 2 decision trees to use in formulation development of poorly soluble drugs. The first step in formulation development, they propose, is to decide whether there is a need for an enabling formulation or a nonconventional (nC) formulation, as they termed it. When developing a formulation, the aim is most often to create the simplest effective formulation; thus, it is usually desirable to use a conventional formulation. Here, a conventional formulation was classed as a formulation using no enabling techniques to improve solubility or permeability but could include drug milling and micronization, adding disintegrating and wetting agents, and granulation. nC formulations were classed into 3 subgroups: solid dispersions, crystalline nanoparticles, and lipidic and surfactant systems. The decision trees were based on a data set of 73 drugs, for which several molecular descriptors were calculated, including but not limited to pK_a , molecular size, hydrogen bond donors and acceptors, polar surface area, solubility, and the number of unmet criteria in Lipinski's rule of 5. A problem-solving method called case-based reasoning was used to build the decision trees. The 2 decision trees made are shown in Figure 7; the first decision tree is used to determine whether there is a need for nC, enabling formulation or if a conventional formulation should be suitable. The second decision tree shows which API attributes are associated with the use of solid dispersions and lipidic and surfactant systems. The numerical values in both decision trees were calculated based on the data set used.

Hariharan et al.³² from the companies Abbott and Pfizer introduced a decision tree for first-in-man formulation development both for parenteral and oral routes (Fig. 8). The tree is divided into 2 main branches, front loaded and non-front loaded, which

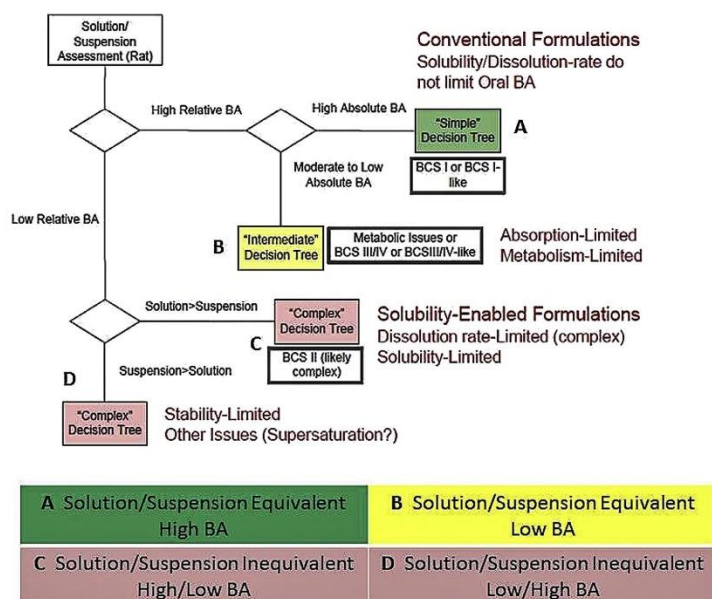


Figure 5. Four possible outcomes of an *in vivo* suspension/solution comparison study are shown. A: If the bioavailability is high for both solution and suspension, then there is no problem with bioavailability. B: However, when both solution and suspension give low bioavailability, the limiting factor is permeability. C: Solution has higher bioavailability than suspension when dissolution rate or solubility is the limiting factor, whereas "D" indicates instability of the drug in solution. Adapted from Brewster.³⁷

correspond to how much time and money is spent on the formulation development in early stages of drug development. Depending on whether a front-loaded formulation strategy is chosen, there are several dosage forms for each delivery route. A non-front-loaded strategy means a shorter time until a clinical trial can be started, choosing a simple formulation only for clinical trials; such strategies are best suited for highly permeable or soluble drugs. Front loading takes more time, developing a formulation which is closer to a marketed formulation. This strategy is well suited for drugs with bioavailability issues, biological drugs, and when drugs show differences in clearance between species.³²

Harris has suggested a general decision tree based on physicochemical properties of the drug that can be used to determine the most suitable formulation for a first-in-man study. It was proposed that this general decision tree can be used for formulation development in later stages and for early clinical formulation development (see Fig. 9).^{12,31}

Mathematical and Statistical Models

Models are very useful tools in formulation development. A model can give a prediction of an outcome for specific experiments, such as stability or dissolution, thus limiting the need for experimentation and reducing both cost and time spent in development. Building a model requires a substantial amount of data and will only be as general as the data set that was used to create it. However, models can in many cases give very accurate predictions and find correlations that are otherwise hard to identify.

Amorphization is an increasingly popular strategy to improve dissolution rates and bioavailability of poorly water-soluble drugs.

The main concern with drugs in amorphous forms is their lack of physical stability and the problem of recrystallization into a more stable, less soluble form. Therefore, the stability of amorphous drugs has been the subject of many studies.^{34–39} If the amorphous stability of an API could be predicted early on in the formulation development, it would be possible to conclude if amorphization were a viable route for an API. Nurzynska et al.⁴⁰ recently developed a predictive model of the amorphous stability of neutral poorly soluble drugs. The statistical model was built based on calculated, predicted, and measured parameters of 25 representative compounds. APIs were rendered amorphous by melting, followed by rapid cooling. When using this method, it is important to ensure that no chemical degradation has occurred.⁴¹ The stability was evaluated over a period of 6 months at a temperature 5 °C below the T_g of each API. Once all the data had been obtained, a model was generated using those parameters that most strongly correlated with the amorphous stability. The best linear-regression model, based on measured, calculated, and predicted parameters, had an R^2 value of 0.82 for prediction accuracy (Eq. 1). Further models were developed using only theoretical parameters but these had lower prediction accuracy.⁴⁰

$$\begin{aligned} \log \text{Stability (days)} = & -0.02 T_m - 0.05 H_f + 0.03 T_g + 0.28 G_c \\ & - 0.01 \tau + 0.23 \text{HBD} - 0.28 \log P \\ & + 0.17 \text{CHA} + 6.42 \end{aligned} \quad (1)$$

The following are the abbreviations used in the model: T_m is the melting temperature in °C, H_f is the enthalpy of fusion in J/g, T_g is

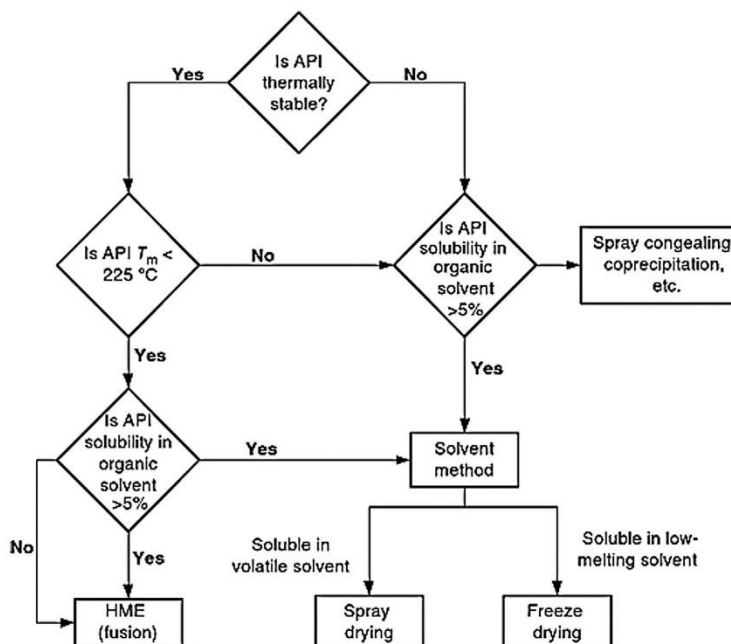


Figure 6. This example decision tree can be used to determine which processing method is most suitable for an API in the development of amorphous solid dispersions.³³

the glass transition temperature in °C, G_c is the configurational free energy in kJ/mol, τ is the relaxation time in hours, HBD is the number of hydrogen bond donors, $c \log P$ is the lipophilicity, and CHA is the ratio of carbons to heteroatoms.

This is the first model to successfully predict long-term amorphous stability of APIs. This study shows that it is

possible to build successful predictive models to help with formulation development. This model could indicate whether an amorphization process would be feasible for an API in development, based on only predicted, calculated, and easily measured parameters that would be available early in the development process.

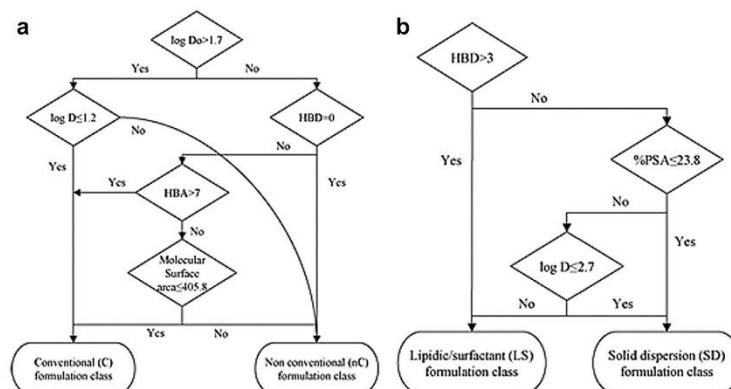


Figure 7. (a) Decision tree used to aid the decision of whether there is a need for an enabling formulation, a nonconventional formulation, or if a conventional formulation would be suitable. This is decided based on the following attributes of the selected API, the dose (log Do), log D, number of H bond acceptors (HBA) and donors (HBD) and the molecular surface area. (b) Decision tree for choosing between a lipid formulation and a solid dispersion formulation. The physicochemical properties used to determine whether a solid dispersion or a lipid formulation is more suitable are HBD, percentage polar surface area (%PSA), and log D. Figure taken from Branchu et al.¹¹

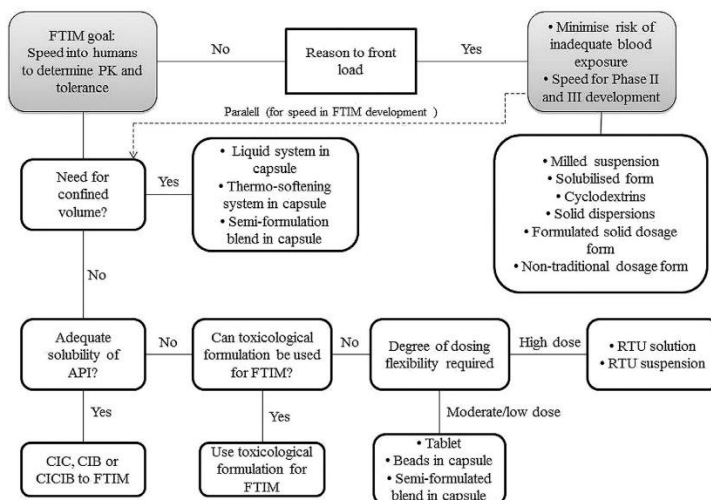


Figure 8. A decision tree for support in first-in-man formulation development branches into 2 main strategies, front loaded and non-front loaded. The decision tree can be used for both oral and parenteral formulations and offers several different formulation types for each. Abbreviations used: CIC, chemical in capsule; CIB, chemical in bottle; CICIB, chemical in capsule in bottle; FTIM, first time in man; RTU, ready to use.

With a similar aim, Mahlin and Bergström¹⁰ built 2 models, one predicting glass-forming ability of APIs and the other predicting the physical stability of the glass formers. A set of 50 structurally diverse drugs was used in the study. The APIs were processed with both melt cooling and spray drying and were divided into glass formers or non-glass formers depending on their ability to form a

glass, which is the amorphous state. The APIs in the glass-forming group were then subjected to a short term, dry stability study, where they were kept in a desiccator at room temperature for 1 month. From the data, a rule of thumb was devised that suggested that drugs with a molecular weight (M_r) >300 transform into the amorphous state using standard processing techniques. A model

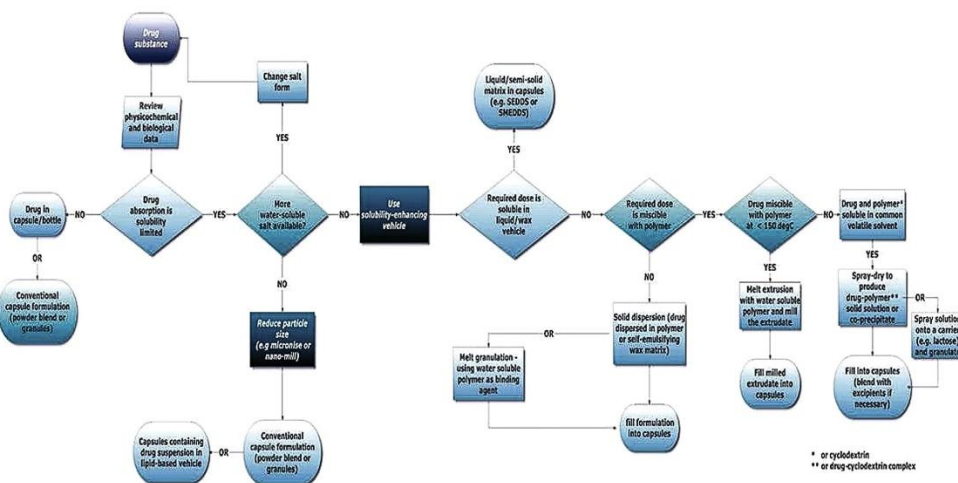


Figure 9. A good general decision tree based on physicochemical properties of the selected drug. The decision tree was aimed for first-in-man formulation but could also be used in later stages of formulation development. Taken from Harris.¹¹

was then generated which correctly predicted the dry amorphous stability of 78% of the glass-forming drugs using only 2 easily calculated or predicted descriptors, T_g and M_n .¹⁰

Further studies which aim to predict glass-forming ability and stability include the work of Alhalaweh et al.⁴² This study confirmed the rule of thumb which Mahlin and Bergström previously proposed that drugs with $M_r > 300$ are glass formers (classes II and III); it was also observed that drugs with $M_r < 200$ were non-glass formers or class I according to Baird et al.³⁴ It was not possible to distinguish between class II (nonstable glass formers) and class III (stable glass formers) just using M_r . However, Alhalaweh et al.⁴² successfully built a model that predicted the correct class of compounds in classes II and III with 78% and 69% accuracy, respectively.

Medarevic et al.⁴³ recently used a combination of experimental design and artificial neural networks to create a model which can predict the dissolution of ternary solid dispersions with varying ratios of carbamazepine and 2 polymers, Soluplus and Poloxamer 188. The created model has a good predictive power, and such a model could be of great value in the formulation development of an immediate-release solid dispersion products.

Mathematical models have been used to predict drug miscibility in polymers. Good miscibility in polymer carriers is pivotal for obtaining sufficient stability for solid dispersions. By using prediction, the most likely polymer can be chosen at the start of the formulation development without the need to test several mixtures. Flory–Huggins theory proposes an equation for calculating the free energy of mixing of a drug–polymer binary system (Eq. 2). Miscibility is achieved when the total free energy of mixing is <0 .⁴⁴

$$\frac{\Delta G_m}{RT} = n_d \ln \phi_d + n_p \ln \phi_p + n_d \phi_p X_{dp} \quad (2)$$

The following abbreviations are used: ΔG_m is the Gibbs free energy; R is the gas constant; T the absolute temperature; n_d and n_p are the numbers of moles of drug and polymer, respectively; ϕ_d and ϕ_p are the volume fractions of drug and polymer, respectively. X_{dp} , Flory–Huggins interaction parameter between drug and polymer.

The Flory–Huggins equation and variations of it have been widely used to predict polymer–drug miscibility.^{37,44–46} One such example is from Duarte et al.,⁴⁷ who have built a support tool to aid in the development of a spray-dried amorphous solid dispersion. The model is a partial differential equation based, among others, on the Flory–Huggins equation and diffuse-interface theories such as Cahn–Hilliard. It assesses thermodynamics, kinetics, and evaporation to predict the miscibility and drug-loading range of itraconazole in 3 different polymers. What makes it different from most other such models is that it takes into account the effect of the solvent in the system and its evaporation.

Conclusions

Formulation development can be time, cost, and compound consuming and does not always produce the most suitable formulation for a NCE. Failure to produce a suitable formulation can hold up progression into clinical trials and can lead to poor and erratic pharmacokinetics, which in some cases can result in termination of a project. Therefore, it is vital to find an appropriate formulation for each NCE and ensure that the development program is as quick and straightforward as possible. The process can be streamlined and have an improved chance of a successful outcome by using a support tool to eliminate a substantial proportion of trial and error from the development process. There are several different approaches that can be used when developing a new solid oral formulation as has been described previously;

some are general and can be used for several different drugs, whereas others are quite specific and are only used for a single drug or a single class of drugs. Each approach or support tool differs slightly although they all have the same goal, to decrease the number of experiments, time, and money spent on formulation development and getting the optimal formulation for each NCE. Which support tool is best suited for a particular drug development program will depend on what the desired target product profile should be as many of the support tools are limited to a single type of formulation such as solid dispersion. Each type of approach has its advantages and limitations. Although there are some commonly available support tools, as mentioned previously, presumably many industrial pharmaceutical companies have their own in-house support tools which are not available to others. Therefore, it is a continuing need for support tools for formulation scientists to help make the development process for new medicines more efficient.

At this time, the validation of any one or combination of approaches has yet to be achieved, and addressing this issue is now timely and would represent a valuable step forward in the development of new medicines.

Acknowledgments

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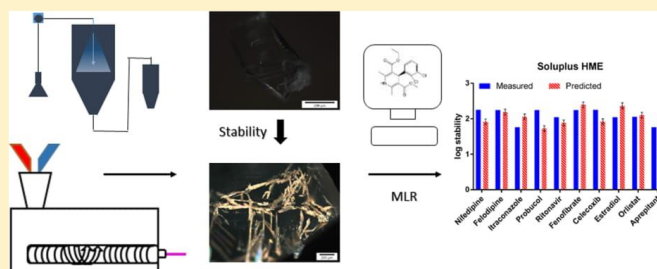
Multiple Linear Regression Modeling To Predict the Stability of Polymer–Drug Solid Dispersions: Comparison of the Effects of Polymers and Manufacturing Methods on Solid Dispersion Stability

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ABSTRACT: Solid dispersions can be a successful way to enhance the bioavailability of poorly soluble drugs. Here 60 solid dispersion formulations were produced using ten chemically diverse, neutral, poorly soluble drugs, three commonly used polymers, and two manufacturing techniques, spray-drying and melt extrusion. Each formulation underwent a six-month stability study at accelerated conditions, 40 °C and 75% relative humidity (RH). Significant differences in times to crystallization (onset of crystallization) were observed between both the different polymers and the two processing methods. Stability from zero days to over one year was observed. The extensive experimental data set obtained from this stability study was used to build multiple linear regression models to correlate physicochemical properties of the active pharmaceutical ingredients (API) with the stability data. The purpose of these models is to indicate which combination of processing method and polymer carrier is most likely to give a stable solid dispersion. Six quantitative mathematical multiple linear regression-based models were produced based on selection of the most influential independent physical and chemical parameters from a set of 33 possible factors, one model for each combination of polymer and processing method, with good predictability of stability. Three general rules are proposed from these models for the formulation development of suitably stable solid dispersions. Namely, increased stability is correlated with increased glass transition temperature (T_g) of solid dispersions, as well as decreased number of H-bond donors and increased molecular flexibility (such as rotatable bonds and ring count) of the drug molecule.

KEYWORDS: solid dispersion, stability, multiple linear regression model, hot melt extrusion, spray-drying, amorphous

INTRODUCTION

Poor water solubility (and hence bioavailability) of drugs in development is an ongoing and challenging problem, as up to 75% of new chemical entities (NCE) in small-molecule drug pipelines are poorly water-soluble.^{1,2} Poor solubility increases the risk of delays and failure in the development of a NCE.³ There are several possible routes that can be taken in order to enhance the solubility and bioavailability of drugs through formulation. These include particle size reduction, polymorph optimization, lipid formulations, and solid dispersions, as reviewed by Williams et al.⁴

Solid dispersions can significantly increase the effective solubility and thus bioavailability of poorly soluble drugs. This can be accomplished by two strategies: particle size reduction

so that the drug is dispersed as tiny particles, or molecular dispersion in a soluble polymeric carrier. The drug is also, if in particulate form, usually dispersed in an amorphous form, which gives increased solubility compared to the crystalline form.^{5–9} As the amorphous form is generally unstable, it is likely to convert to the more stable crystalline form, which in turn reduces solubility. Other processes and factors can also contribute to instability within a solid dispersion, inclusive of phase segregation leading to regions with different composi-

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tions, chemical degradation of the Active Pharmaceutical Ingredient (API) leading to products that may alter crystallization tendencies, and water uptake by the matrix polymer that can affect drug and polymer mobility and hence stability.¹⁰ Physical stability of solid dispersions is therefore the most significant obstacle that must be overcome in their development. Choosing an appropriate polymer and processing method is pivotal in producing a successful product as it will greatly affect the stability of the solid dispersions. The most commonly used manufacturing methods are spray-drying and hot melt extrusion (HME) as these are relatively easy to scale up and produce well mixed dispersions.^{11,12} The chosen polymer carrier stabilizes the drug both in the solid form and in the solid solution, retarding crystallization.^{7,13} Solid dispersions have been extensively studied in this context (reviewed by Leuner and Dressman¹⁴). Solid dispersions have been used successfully to enhance the bioavailability of drugs in several marketed products as summarized in Table 1.

Table 1. Summary of Marketed Solid Dispersions in Oral Dosage Forms, Including the Brand Name, the APIs They Contain, and the Polymer Carriers Used^{7,15,16}

brand name	API	polymer carrier ^d
Adalat SL	nifedipine	PEG
Afeditab CR	nifedipine	poloxamer-PVP
Certican	everolimus	HPMC
Cesamet	nabilone	PVP
Crestor	rovastatin	HPMC
Envarsus	tacrolimus	HPMC
Fenoglide	fenofibrate	PEG
Gris-PEG	griseofulvin	PEG
Incivo	telaprevir	HPMCAS
Intelence	etravirine	HPMC
Isopstin SR-E	verapamil	HPC/HPMC
Kaletra	lopinavir/ritonavir	PVP-VA
Kalydeco	ivacaftor	HPMCAS
Lopinavir/Ritonavir	lopinavir/ritonavir	HPMCAS
Mylan		
Modigraf	tacrolimus	HPMC
Nivadil	nivaldipine	HPMC
Noxafil	posaconazole	HPMCAS
Orkambi	lumacaftor/ivacaftor	HPMCAS
Prograf	tacrolimus	HPMC
Rezulin	trogilazone	HPMC
Sporanox	itraconazole	HPMC
Viekirax	ombitasvir/paritaprevir/ritonavir	PVP-VA
Votubia	everolimus	HPMC
Zelboraf	vemurafenib	HPMCAS

^dThe following abbreviations are used: PEG, polyethylene glycol; PVP, polyvinylpyrrolidone; HPMC, hydroxypropyl methylcellulose; HPMCAS, hydroxypropyl methylcellulose acetate succinate; HPC, hydroxypropyl cellulose.

Choosing the most appropriate formulation route for a new drug is to a large extent still based on formulator know-how and experimental screening assays. Properties of the API are often not considered, but rather in-house knowledge and availability of equipment steer the process.^{17,18} We propose that a knowledge-based computational tool that could predict the best formulation strategy would greatly improve this process. Indeed there are some support tools available that can be used

to facilitate this process such as guidance maps, high throughput screening, and statistical models, as we recently reviewed.¹⁹ Using a support tool in the formulation development can shorten the development process, limiting the number of experiments that need to be carried out by indicating which formulation route is appropriate for a specific API, and there remains a significant need in this area.

As the choice of polymer carrier and processing method are vital to the consequent stability of a solid dispersion, the use of a knowledge-based support tool in the formulation development is very beneficial. Currently, although some effort has been put into building such tools, their availability remains quite limited.^{18,20} Several models have been built to predict the glass-forming ability of APIs, and some predict the stability of the amorphous form of APIs. These are intended to give an indication of whether a dosage form containing the API in amorphous form is a viable formulation choice.^{21–26} Recently molecular dynamics (MD) simulations have been used to predict T_g and investigate interactions between drug and polymer in solid dispersions and the effect of water in the system.²⁷ Historically the Flory–Huggins equation or Hansen solubility parameters have also been used theoretically to analyze interactions and miscibility and provide an indication of likely stability. The advancement of a computational tool that can compare several solid dispersion formulations and predict their stability has not yet been achieved.

Many researchers have focused on the stability of solid dispersions in their studies, in particular which factors inhibit crystallization.^{28–31} With better knowledge of which factors are important for stabilization, such as drug–polymer interaction, polymer T_g , and hygroscopicity, formulation development of solid dispersions could be improved. This in turn could ease the path of such new formulations to the market. However, although many drugs and polymers have been researched, the number of APIs in such studies is often very limited. This, along with the fact that there is little agreement in results between different studies, makes it difficult to draw any general conclusions, as has been noted before.³² Therefore, the aim of the present study is to produce and test the stability of a large number (60) of solid dispersions using chemically diverse APIs (10), commonly used polymers (3), and well-known processing methods, spray-drying and melt extrusion. This approach should yield more general conclusions about important factors for solid dispersions' physical stability. The data set was then used to build a statistically predictive model that should be able to highlight which physicochemical properties of the APIs are correlated with formulation stability. The models could then be used to facilitate formulation development of solid dispersions by indicating which combination of polymer and processing method is most suitable for a new drug.

MATERIALS AND METHODS

Ten poorly soluble, neutral ($pK_{a\text{acid}} > 8.5$ and $pK_{a\text{basic}} < 4.5$), chemically diverse APIs (purity 99% unless otherwise indicated), namely, nifedipine (1), felodipine (2), itraconazole (3), probucol (4), ritonavir (5), fenofibrate (6), celecoxib (7), estradiol (8), orlistat (9), and aprepitant (10) (purity 98%), see Figure 2, were purchased from Sequoia Research Products Ltd. Three polymers were generous gifts from their commercial suppliers: Soluplus (PCL-PVAc-PEG, SOL) and Kollidon VA64 (PVP-VA) from BASF, AquaSolve (HPMCAS-HG) from Ashland (Figure 1). The molecular weight distribution of

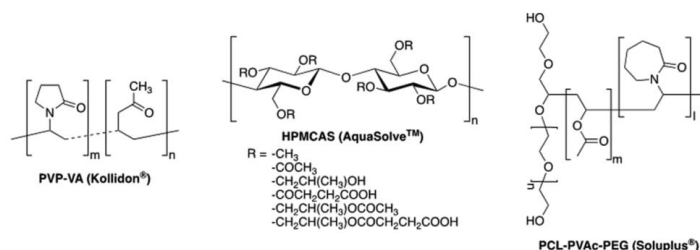


Figure 1. Chemical structure of PVP-VA (P), HPMCAS (H), and SOL (S).

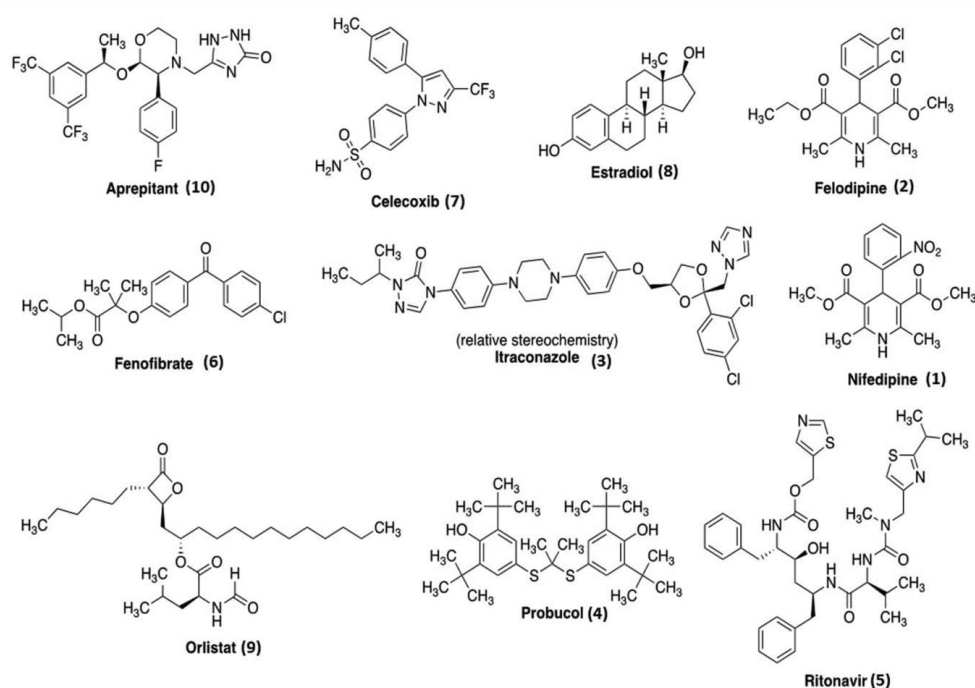


Figure 2. Chemical structures of the ten APIs used in this study.

the three polymers was as follows: 45,000–70,000 Da for PVP-VA, 55,000–90,000 Da for HPMCAS, and 90,000–140,000 Da for SOL. Binary solid dispersions were made for each combination of polymer and drug. Each combination was produced twice, once using hot melt extrusion (HME) and once by spray-drying. Each formulation was then subjected to a stability test and characterized at each time point. The following abbreviations of formulations are used below: numbers one to ten for the APIs as listed above (Figure 2); for the polymers, S, P, and H for Soluplus, PVP-VA, and HPMCAS, respectively; and hot melt extrusion (h) and spray-drying (s). Thus, e.g., spray-dried ritonavir–PVP-VA is abbreviated SPs.

Hot Melt Extrusion. A HAAKE minilab II micro compounder (Thermo Fisher Scientific) was used for the production of the HME solid dispersions. All formulations were

made with 10% w/w drug loading in polymer. In order to keep the number of variables as low as possible, all solid dispersions were produced using the same method and no optimization was performed for any formulation. API and polymer were weighed and mixed in a scintillation vial. Once the compounder had reached the set temperature of 160 °C, the mixture was fed into the hopper. The twin screws were corotated at 50 rpm and the mixture was circulated for 15 min before being extruded. The same method was used for all formulations except those containing orlistat, which was not circulated but extruded immediately because of its low melting point (44 °C). The processing temperature of 160 °C was selected as an optimal value suitable for the processing of all the selected materials and one that did not cause degradation of the materials during the exposure to this temperature. This processing temperature is above the T_g of all three polymers. Therefore, for the three

Table 2. Calculated and Predicted Parameters Used in the Building of the MLR Models

physicochemical property	nifedipine	felodipine	itraconazole	probucol	ritonavir	fenofibrate	celecoxib	estradiol	orlistat	aprepitant
nAliph	19	19	21	23	28	13	9	14	35	20
nAliphRing	1	1	2	0	0	0	0	3	1	1
nAr	6	6	28	12	22	12	17	6	0	17
nArRing	1	1	5	2	4	2	3	1	0	3
ASA ($\text{\AA}^2$)	533	583	976	874	981	642	602	392	985	679
nAtom	43	44	87	83	98	46	40	44	88	58
avPol ($\text{\AA}^3$)	33	37	74	62	76	38	35	32	61	44
nBond	44	45	93	84	101	47	42	47	88	61
nChir	0	1	3	0	4	0	0	5	4	3
HBA	5	3	9	2	6	3	3	2	3	6
HBD	1	1	0	2	4	0	1	2	1	2
nHet	8	7	14	4	13	5	9	2	6	14
HLB	16	11	5	2	4	4	4	2	3	16
logS ₀ (M)	−3	−4	−9	−11	−8	−6	−6	−4	−11	−6
logP	1.82	3.44	7.31	10.57	5.22	5.28	4.01	3.75	8.11	4.52
MW	346.3	384.3	705.6	519.8	721.0	360.8	381.4	272.4	495.8	534.4
molPol ($\text{\AA}^3$)	33	37	71	61	75	38	36	31	56	43
mp ($^{\circ}\text{C}$)	173	145	166	125	121	81	158	152	44	255
nC	17	18	35	31	37	20	17	18	29	23
nCl	0	2	2	0	0	1	0	0	0	0
nF	0	0	0	0	0	0	3	0	0	7
nN	2	1	8	0	6	0	3	0	1	4
nO	6	4	4	2	5	4	2	2	5	3
nS	0	0	0	2	2	0	1	0	0	0
nRing	2	2	7	2	4	2	3	4	1	4
nRot	6	6	11	8	18	7	4	0	23	8
sp ²	12	12	22	12	21	14	15	6	3	14
sp ³	5	6	13	19	16	6	2	12	26	9
b.pKa	−6.63	−6.64	3.92	−5.08	2.84	−4.93	0.08	−0.88	−1.44	3.74
tPSA ($\text{\AA}^2$)	107.8	64.6	100.8	40.5	145.8	52.6	78.0	40.5	81.7	75.2
vdW-SA ($\text{\AA}^2$)	466	493	965	916	1,065	533	484	437	925	656
Sh $T_{g,meas}$ ($^{\circ}\text{C}$)	70.2	68.2	68.5	68.9	66.6	56.1	72.7	64.2	NA	74.3
Ss $T_{g,meas}$ ($^{\circ}\text{C}$)	71.2	67.8	70.0	67.0	68.3	57.5	71.7	68.0	NA	74.2
Kh $T_{g,meas}$ ($^{\circ}\text{C}$)	102.3	100.9	103.6	101.4	96.3	84.8	104.8	103.1	NA	106.0
Ks $T_{g,meas}$ ($^{\circ}\text{C}$)	104.8	104.5	109.9	103.4	102.5	91.8	103.7	99.5	NA	110.6
Hh $T_{g,meas}$ ($^{\circ}\text{C}$)	105.3	103.4	109.0	101.7	97.6	92.7	102.0	104.7	NA	NA
Hs $T_{g,meas}$ ($^{\circ}\text{C}$)	103.7	103.7	109.3	104.3	102.6	91.1	103.7	100.0	94.8	110.0
S $T_{g,pred}$ ($^{\circ}\text{C}$)	67.5	67.5	68.8	65.7	67.9	61.1	68.8	71.5	58.5	66.6
P $T_{g,pred}$ ($^{\circ}\text{C}$)	95.4	95.4	96.7	93.6	95.8	89.0	96.7	99.4	86.4	94.5
H $T_{g,pred}$ ($^{\circ}\text{C}$)	112.5	112.5	113.8	110.7	112.9	106.1	113.8	116.5	103.5	111.6

APIs with melting points above 160 $^{\circ}\text{C}$, as long as they are miscible in the polymer, they should dissolve in the polymer and be fully amorphized and mixed in the solid dispersion. The success of this approach, or not, would be apparent from studies of the melt extruded mixtures. These did not result in markedly less stable formulations than those APIs with lower mp. Once extruded, the formulation was placed into amber HPLC vials and stored at 5 $^{\circ}\text{C}/0\%$ RH until the start of the stability study.

Spray-Drying. As with the HME formulations, to keep the number of variables at a minimum, the same method was used to produce all formulations. All were produced using the same solvent, except for itraconazole, which was not soluble in methanol and was therefore dissolved in dichloromethane. Spray-drying was performed using a Büchi B290 instrument. Each drug–polymer mixture was dissolved in methanol at 10% w/v and spray-dried within half an hour. Once the mixture was fully dissolved in the solvent, the solution was sprayed through the nozzle using an inlet temperature of around 80 $^{\circ}\text{C}$ to

maintain an outlet temperature of 50 $^{\circ}\text{C}$. The produced formulation was then collected and weighed before being placed in amber HPLC vials and stored at 5 $^{\circ}\text{C}/0\%$ RH until the stability study was started.

Stability. A six-month stability study was conducted on all produced solid dispersions at accelerated conditions, 40 $^{\circ}\text{C}/75\%$ RH, in accordance with ICH guidelines.³³ The samples were kept in glass vials, open containers, and conditions were monitored using a Testo 174H temperature and humidity logger. The amorphicity of the formulations was confirmed at the start of the stability study. Subsequently the solid dispersions were analyzed at 12 h, 7 d, 1 month, 2 months, 4 months, and 6 months. For those solid dispersions that were still amorphous after 6 months, a 1-year time point was added. At each of these time points the samples were examined for signs of crystallinity using polarized light microscopy (PLM) as a sensitive method to detect onset of crystallization. Any formulations with signs of crystallinity by PLM were then further characterized using differential scanning calorimetry

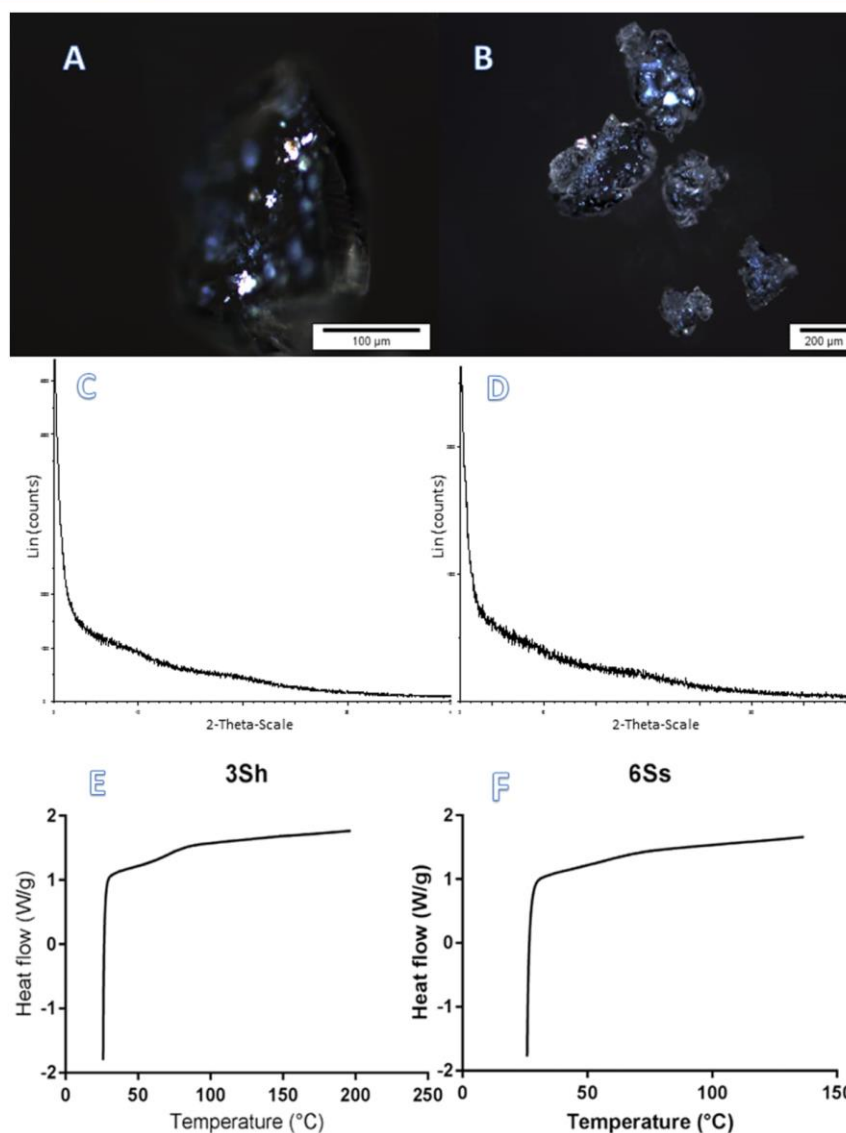


Figure 3. (A) 3Sh with clearly visible crystals under polarized light; (C, D) no melting peak is visible under DSC nor any peaks in XRPD. (B) 6Ss with abundance of crystals visible, again no melting peak by DSC nor peaks indicating crystallinity in XRPD (D, F).

(DSC) and X-ray powder diffraction (XRPD). Before analyzing the samples at each time point the formulation was ground in a mortar, if necessary, producing small glass-like pieces appropriate for characterization with PLM, DSC, and XRPD.

Polarized Light Microscopy. An Olympus BX51 optical microscope fitted with polarizing filters and equipped with an

Olympus DP71 camera was used visually to check for presence of any crystalline material. A small amount of powder (spray-dried) or ground glass (HME) was placed on a microscope slide with a glass coverslip. The samples were examined using both cross-polarized and nonpolarized light. Images of identical frames were recorded for each sample under both polarized and

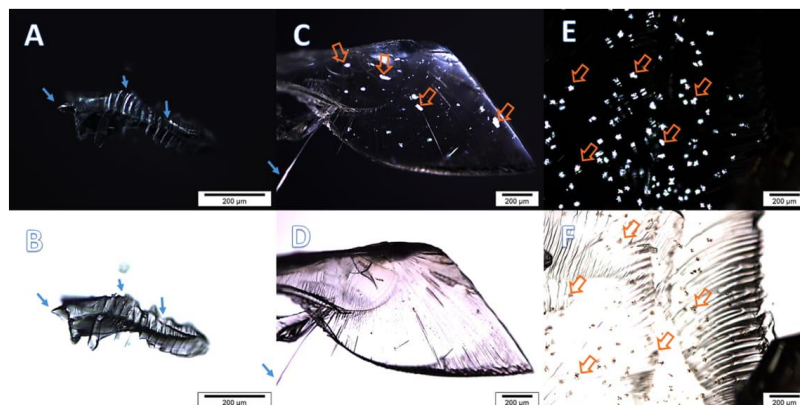


Figure 4. Pieces of HME formulations under polarized light (A, C, and E) and nonpolarized light (B, D, and F). (A) 8Ph sharp edges (blue, thin arrows) are visible under polarized light; the visible lines are confirmed as part of the glass rather than crystals (B). Panels C and D show visible crystals (orange, thick arrows) under polarized light, 9Hh and 8Sh, respectively. No sign of crystals can be seen under normal light in D, but crystals are clearly observed in F.

nonpolarized light. Cell[^]F imaging system from Olympus was used to acquire images of the samples.

Differential Scanning Calorimetry. A Q2000 (TA Instruments) was used for the DSC measurements of all samples, using Tzero pans (TA Instruments) and pierced lids. The instrument was calibrated against indium. Approximately 5 mg of sample was weighed into each pan. Each sample was run in duplicate. All solid dispersions, except orlistat, were run as a cycle where the first ramp was used to remove water and solvent from the formulation. The second time the sample was ramped up to 20 °C above the melting point of the drug. Both up and down ramps were performed at 10 °C/min. As orlistat has a melting point of 44 °C it was not feasible to go above 100 °C in order to remove water before analyzing the sample as this might have affected the solid dispersion. It was therefore run as a single ramp. The results were analyzed using the TA Universal Analysis program to determine T_g and melting points (mp).

X-ray Powder Diffraction. A Bruker D8 Advance instrument using a Cu $K\alpha_1$ radiation source and equipped with an incident beam monochromator was used for the XRPD measurements. The instrument was calibrated against corundum. All samples were analyzed with spinning at a current of 40 mA and a voltage of 40 kV using standard scanning parameters, i.e., from 2 to 40° (2 θ) using a step size of 0.02° and a time per step of 1 s. A zero-background holder was used for the measurements of samples of approximately 3–5 mg. The program DIFFRAC.SUITE EVA from Bruker was used to analyze and plot the data, and no adjustments were done to the baseline during plotting.

Physicochemical Properties of APIs. The program Instant JChem from ChemAxon was used to calculate and predict physicochemical properties of the APIs. These were based on the molecular structure of each API. Furthermore, information on the melting point (mp) of each drug was obtained from Chempider, these did not differ significantly from measured mp.³⁴ The following parameters were included in the modeling process: molecular weight (MW), lipophilicity

(ClogP) determined using the ChemAxon method, strongest basic pK_a (b.pKa), topological polar surface area (tPSA), number of atoms (nAtom), number of bonds (nBond), number of chiral atoms (nChir), number of H-bond acceptors (HBA), number of H-bond donors (HBD), number of rings (nRing), number of nonterminal rotatable bonds (nRot), number of aliphatic atoms (nAliph), number of aromatic atoms (nAr), water accessible surface area (ASA), average polarizability (avPol), hydrophilicity–lipophilicity balance (HLB), intrinsic water solubility in log₁₀ of molar solubility (logS₀), molecular polarizability (molPol), van der Waals surface area (vdW-SA), melting point (mp), number of aliphatic rings (nAliphRing), number of aromatic rings (nAr), number of carbon atoms (nC), number of fluorine atoms (nF), number of nitrogen atoms (nN), number of oxygen atoms (nO), number of sulfur atoms (nS), number of chlorine atoms (nCl), number of heteroatoms (atoms other than carbon and hydrogen; nHet), number of sp³ carbon atoms, number of sp² carbon atoms, measured T_g ($T_{g,meas}$), and predicted T_g ($T_{g,pred}$) in °C, where the Fox equation was used to calculate the expected T_g of the solid dispersion. The predicted value was also used as a $T_{g,meas}$ in those instances where a T_g could not be measured. The physicochemical properties used and their values are listed in Table 2.

Multiple Linear Regression (MLR) Model. The publicly available software R³⁵ and R studio (version 1.0.143) were used to build the multiple linear regression models. The calculated and predicted parameters (see Table 2) were used without units as input variables in the model equations. The measured stability (time to crystallization), the output, was used as the log₁₀ value, stability in days +1 in order to avoid zero values. Using the leaps package in R an exhaustive selection of covariates was made with one, two, and three as the maximum number of covariates, using backward, forward, stepwise, and sequential replacement. This was done to ensure that the simplest, most effective model could be made, so that each parameter improves the model more than can be expected by chance. To avoid linear dependency of covariates, the selected

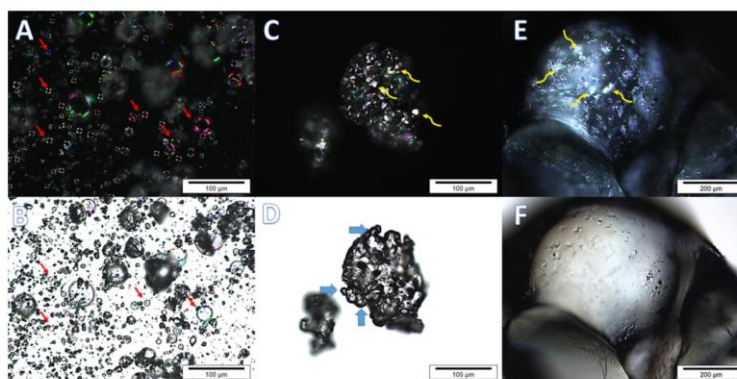


Figure 5. Panels A, C, and E show solid dispersions under cross-polarized light while panels B, D, and F show the same samples imaged under normal light. Panels A and B show a well-dispersed powder, 6Hs, where typical bubble-like particles (red, thin arrows) can be observed. Panels C and D show a piece of 6Ps where the powder has transformed into glass. It is possible to still see signs of the original particles that have merged (blue, thick arrows). Panels E and F show a large piece of 6Ss formulation. Here the transformation into glass is more complete than in panel D as no signs of previous particles can be observed. Crystallization can be observed (yellow, curly arrows) in C and E.

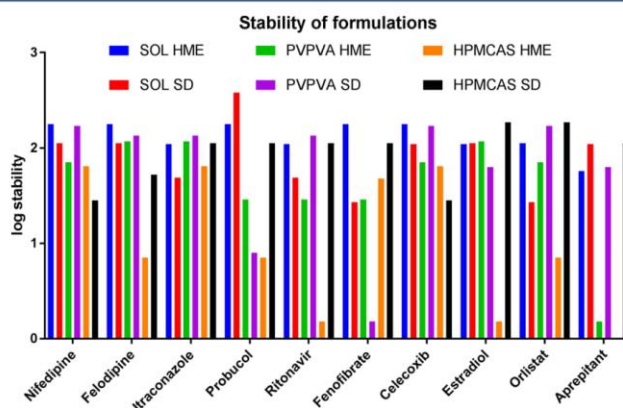


Figure 6. Measured stability of the solid dispersions (last time point where no crystals were observed plotted as $\log(\text{stability})$).

parameters were tested for intercorrelation, and further iterations of parameter selection were made if this was found to be higher than 0.9. Once three linearly independent parameters had been selected, these were used to build a model that linked the physicochemical properties with the logarithm of the measured stability, i.e., time until onset of crystallization. As this was a relatively limited data set (in terms of statistical analysis) of ten observations per model, the whole data set was used to build the models, rather than a training and test set method. A leave-one-out cross validation (LOOCV) was performed on each model to test its predictability and to calculate the mean square error (MSE). This process was repeated for each data set (combination of polymer and processing method), resulting in six MLR models.

RESULTS

Due to limit of sensitivity of XRPD and DSC, PLM was used as the main method of detecting onset of crystallization, while further crystallization was confirmed using the inherently more quantitative XRPD and DSC methods. In many cases, even when an abundance of crystals was observed by PLM, no crystallinity could be detected with either XRPD or DSC (Figure 3).

Stability. For each API–polymer mixture the HME solid dispersion appeared as a clear glass after extrusion, an indication of amorphicity, except 10Hh, which was cloudy. At the start of the stability assessment all the clear glass HME formulations and all the spray-dried formulations for all APIs were confirmed to be amorphous, while the cloudy 10Hh formulations were confirmed to contain crystals. Crystals were defined as bright white or colored spots under polarized light. In each case it was ascertained that spots in the PLM images

displayed a distinctive pattern that was not due to any physical defects of the surfaces of the examined samples, such as cracks and sharp edges (Figure 4). Even though the onset of crystallization was the main outcome of this study, once a formulation showed signs of crystallinity, during the stability study, it was followed up at each time point after onset to confirm that further crystallization had occurred and that the first onset point was not an artifact of measurement.

At the start of the stability study, the spray-dried solid dispersions were dispensed into the storage vials as dry dispersible powders, but after a period stored at 40 °C/75% RH, most of the powders had converted to solid glasses, similar in appearance to the HME glass. In most cases signs of crystallization were not seen until after the transformation of the powder to the glass. How fast this transformation was observed depended on both the polymers and the APIs as some solid dispersions were more resistant. The first transformation was seen after one month at accelerated conditions for 5Ss, 6Ss, and 9Ss. Most appeared after two months and a few after four months, 3Ps, 7Ps, and 10Ps. The only formulation not to show any signs of transformation during the one-year stability study, of those containing PVP-VA and SOL, was 4Ss. An example of this transformation can be seen in Figure 5 where the first image shows a well-dispersed powder; panels C and D show a piece where signs of merged particles can be seen, but in the last image no signs of previous particles are observed. This transformation to glass was not observed under less accelerated conditions and not for any of the HPMCAS spray-dried formulations.

The onset of crystallization was noted for each formulation. For those solid dispersions where no signs of crystals could be found after 6 months at 40 °C/75% RH a 1-year time point was added. In Figure 6 the stability, i.e., the last time point where no signs of crystals were observed, is summarized for each API–polymer combination. For example, if crystals were first observed at 4 weeks, crystals must have started growing between time points 1 week and 4 weeks. Thus, the last data point of confirmed amorphicity is 1 week and that is the noted stability.

An overview of the difference in stability is provided in Figure 6. Significant differences in stability between the polymers can be observed for some APIs. As an example, the last amorphous time point for felodipine was observed as 1 week in the HPMCAS HME formulation, at 16 weeks when formulated with PVP-VA by HME, and at 24 weeks with SOL by HME. A similar example can be found in the spray-dried formulations; probucol was only stable for 7 days in PVP-VA, was stable for a moderate 111 days in HPMCAS, but was still amorphous at the one-year point (379 days) in SOL. Some similarities are also observed between the polymers; aprepitant seems to be the least stable of all the drugs by HME regardless of the polymer used, although the onset of crystallization time varies from zero to 16 weeks. Additionally, differences were observed between the two methods; spray-dried formulations seem to be more stable for PVP-VA and HPMCAS while the opposite is true for SOL formulations.

MLR Models. Six statistical MLR models were constructed, one for each combination of polymer and manufacturing method (Table 3). Even though each modeling process started with the same predicted, calculated, or measured parameters, none of the models were identical, each indicating that different parameters were influential in the stability of that particular polymer dispersion and manufacturing method. This provides a

Table 3. An Overview of the Abbreviations Used for the Different Polymer-Manufacturing Method Combinations

abbreviation	formulation
Sh	SOL HME
Ss	SOL spray-dried
Ph	PVP-VA HME
Ps	PVP-VA spray-dried
Hh	HPMCAS HME
Hs	HPMCAS spray-dried

direct indication of why it has been difficult to find agreement among different studies on the most important parameters to consider when seeking the most stable solid dispersion formulation and production method.

Due to the relatively limited data set (although very large compared to previous studies), the number of independent physicochemical parameters in each model was limited to three. Models containing one and two parameters were also built as well as models containing pooled data sets. Additionally, there is a greater risk of overfitting when using small data sets, which is why leave-one-out validation was used to explore the general features of the data. The first step was determining which three parameters had the strongest correlation with the stability of the formulations.

Once the three most influential parameters had been chosen, a MLR model was derived using the stability of the ten drugs and the parameters. The models, adjusted R^2 (R^2_{adj}), and mean square error are listed in Table 4. The Sh stability model shows a negative correlation with the number of H-bond acceptors and the number of chiral atoms, and a positive correlation with topological polar surface area. If number of F atoms was included in the model, the adjusted R^2 was 0.918, and the MSE was 0.08, the model was then $\log(\text{stability}) = 2.675 - 0.04 \text{ nF} - 0.132 \text{ nAliphRing} - 0.001 \text{ MW}$. Ss formulation stability is positively correlated with measured glass transition temperature ($T_{g, \text{meas}}$) and molecular polarizability while negatively correlated with the number of N atoms in the drug. Stability of Ph shows a positive correlation with predicted glass transition temperature ($T_{g, \text{pred}}$) and a negative correlation with number of H-bond donors and the melting point of the API. The nitrogen count and $T_{g, \text{pred}}$ are positively correlated with stability of Ps while the number of aromatic atoms shows a negative correlation. The Hh stability shows negative correlation with number of HBD and lipophilicity while sulfur count is positively correlated. Both ring count and number of rotatable bonds are positively correlated with the stability of HPMCAS spray-dried, but the number of nitrogen atoms is negatively correlated. The optimal models have R^2_{adj} values between 0.798 and 0.895.

The MSE was calculated from a leave-one-out validation of each model. In a leave-one-out cross validation (LOOCV) one of the data points (formulations) is excluded from the data set used to build the model. The same procedure is used as with the whole data set, and the parameters are selected before the model is built. For example, a model was built leaving out the nifedipine data. The new model was then used to predict the stability of the drug that was excluded, nifedipine. This was repeated ten times, each time excluding one API and predicting the stability of that API. The MSE of these predictions was then calculated, and it gives an indication of the predictability of the models. The comparison of predicted and measured stability from each LOOCV can be seen in Figure 7. Substantial difference in MSE can be seen between the models (see both

Table 4. Summary of Models, Including Adjusted R^2 and MSE Values^a

name	nCov	model	R^2_{adj}	MSE
Sh	3	$\log \text{Stab} = 2.307 + 0.003 \text{ tPSA} - 0.055 \text{ nChir} - 0.082 \text{ HBA}$	0.891	0.08
Ss	3	$\log \text{Stab} = -3.864 + 0.081 T_{g, \text{meas}} - 0.163 \text{ nN} + 0.015 \text{ molPol}$	0.895	0.265
Ph	3	$\log \text{Stab} = -11.2106 - 0.2355 \text{ HBD} - 0.0112 \text{ mp} + 0.1536 T_{g, \text{pred}}$	0.815	1.021
Ps	3	$\log \text{Stab} = -5.252 + 0.079 T_{g, \text{pred}} + 0.390 \text{ nN} - 0.119 \text{ nAr}$	0.798	2.113
Hh	3	$\log \text{Stab} = 2.472 - 0.849 \text{ HBD} - 0.124 \text{ ClogP} + 0.771 \text{ nS}$	0.827	3.481
Hs	3	$\log \text{Stab} = 0.953 + 0.055 \text{ nRot} + 0.331 \text{ nRing} - 0.216 \text{ nN}$	0.870	0.781
Sh	2	$\log \text{Stab} = 1.988 + 0.078 \text{ nS} - 0.042 \text{ pKa}_b$	0.781	0.068
Ss	2	$\log \text{Stab} = -1.829 - 0.059 \text{ nHet} + 0.063 T_{g, \text{meas}}$	0.647	0.308
Ph	2	$\log \text{Stab} = -0.605 - 0.232 \text{ nF} + 0.025 T_{g, \text{meas}}$	0.651	1.517
Ps	2	$\log \text{Stab} = 2.116 - 0.104 \text{ nAr} - 0.389 \text{ nN}$	0.621	1.052
Hh	2	$\log \text{Stab} = 1.754 - 0.711 \text{ HBD} + 0.480 \text{ nS}$	0.688	2.619
Hs	2	$\log \text{Stab} = 1.415 + 0.060 \log P + 0.102 \text{ nChir}$	0.700	0.276
Sh	1	$\log \text{Stab} = 2.033 - 0.042 \text{ pKa}_b$	0.659	0.029
Ss	1	$\log \text{Stab} = -0.667 + 0.038 T_{g, \text{meas}}$	0.272	0.392
Ph	1	$\log \text{Stab} = 1.831 - 0.199 \text{ nF}$	0.605	0.394
Ps	1	$\log \text{Stab} = 0.839 + 0.012 \text{ tPSA}$	0.231	7.445
Hh	1	$\log \text{Stab} = 1.669 - 0.479 \text{ HBD}$	0.534	0.520
Hs	1	$\log \text{Stab} = 1.599 + 0.030 \text{ sp}^3$	0.483	0.149

^aThe following abbreviations are used: nCov, number of covariants; R^2_{adj} , adjusted R^2 ; MSE, mean square error; log Stab, log stability; tPSA, topological polar surface area atoms; HBA, number of H bond acceptors; mp, melting point; nF, number of F atoms; nS, number of S atoms; nN, number of N atoms; HBD, number of H bond donors; nRot, number of rotatable bonds; molPol, molecular polarizability; $T_{g, \text{pred}}$, predicted T_g using the Gordon–Taylor equation; $T_{g, \text{meas}}$, measured T_g ; nRing, number of rings; nAr, number of aromatic atoms; nHet, number of heteroatoms; sp^3 , number of sp^3 carbon atoms.

Figure 7 and Table 4); the lowest MSE is in the Sh model, 0.08, while the highest is seen in the Hh model, 3.481.

Models were also made using only one or two parameters (Table 4); these had lower R^2_{adj} , 0.231–0.659 and 0.621–0.781, respectively, but in most cases lower MSE, 0.029–7.445 and 0.068–2.619. Attempts to build models using pooled data were unsuccessful, with R^2_{adj} ranging from -0.1 to 0.33 . These included six pooled data sets, one including all data, one for each method, containing data from all polymers, and one for each polymer which included both methods.

A further external validation was performed. A recent study compared the stability of three solid dispersion formulations of lapatinib (structure Table 5), namely, SOL, PVP-VA, and HPMCAS. These were spray-dried at 40% w/w API and stored at 40 °C/75% RH. It was found that the formulation containing HPMCAS was the most stable by far, staying amorphous for the six months tested while both PVP-VA and SOL formulations were crystalline by one month.⁵⁶ Using our Ss, Ps, and Hs models, the stability of lapatinib formulations was predicted in each polymer. The models ranked the stability correctly using both two- and three-parameter models. For the three-parameter models the stability was less than one month for SOL and PVP-VA and more than six months for HPMCAS, reproducing perfectly the measured stability (Table 5).

DISCUSSION

Most studies considering the stability of solid dispersions solely rely on DSC and XRPD for detecting crystals, not PLM.^{7,37–40} However, it has been observed in this study that specifically at

low drug loading these two methods lack the sensitivity to detect onset of crystallinity accurately. Here PLM has therefore been used as a primary method of detection as it is known to be a sensitive and rapid method to detect small amounts of crystalline material in amorphous matrices.^{41–43}

Stabilizing Polymer. For some drugs there is a clear difference in stability between polymers while for others there is minimal difference. For instance probucol was very stable in both formulations containing SOL. No crystals were observed up to six months in HME, and it was still amorphous after one year in spray-dried formulations. Probuco was the only drug that stayed amorphous in spray-dried SOL for more than four months and the only formulation to stay amorphous for the whole year tested. However, for PVP-VA it was one of the first APIs to show signs of crystallization in both methods, 4 and 8 weeks, respectively for spray-dried and HME. In HPMCAS the crystallization onset was determined to be 4 weeks for HME and 24 weeks for spray-dried. When considering the physicochemical properties of probucol, ClogP stands out as extreme. Probuco has a ClogP of 10, which is the highest of the APIs used in this study. It is possible that this attribute is connected with this difference in stability, and the PEG and PCL parts of SOL may better stabilize this highly lipophilic molecule compared to the more hydrophilic PVP-VA, and the amphiphilic HPMCAS.⁴⁴ Other drugs such as fenofibrate started crystallizing at the same time for all three polymers, at 8 weeks for HME HPMCAS and PVP-VA and spray-dried SOL.

When comparing the stability of spray-dried solid dispersions it is interesting to note that, for each of the polymers, two APIs had shown signs of crystallization by two months. However, out

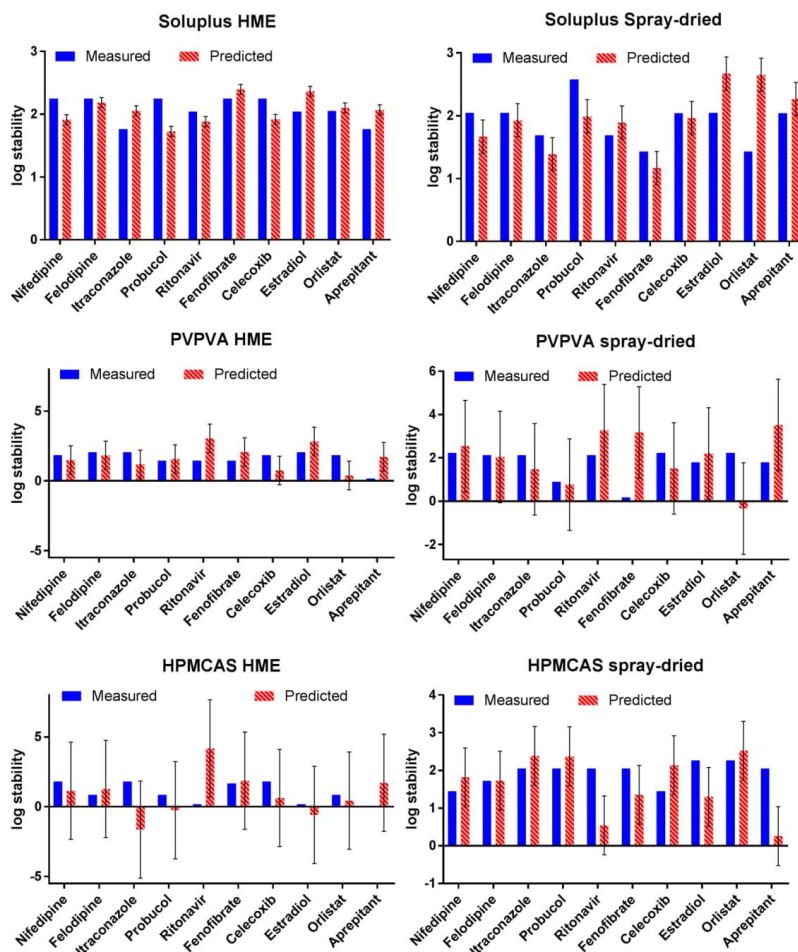
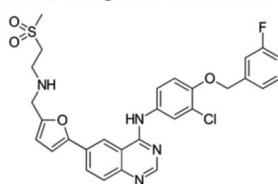


Figure 7. Leave-one-out cross validation of each of the six models. The error bars on the predicted stability are the MSE of each model.

Table 5. Measured³⁶ and Predicted Stability (Using Three Covariant Models) of Lapatinib Formulations^a

	SOL	PVP-VA	HPMCAS
measured	<1 month	<1 month	>6 months
predicted	19 days	0 days	220 days

^aThe molecular structure of lapatinib is shown above the table.



of the six crystallized formulations only two contained the same drug. For HPMCAS it was celecoxib and nifedipine; probucol and fenofibrate for PVP-VA; and orlistat and fenofibrate for SOL. It can therefore be assumed that this difference is due to the stabilizing effect of the polymer or a lack thereof. Even the least stable drugs behave quite differently depending on the polymers. This would indicate that relative stability is not solely due to the method used or the inherent instability of the API, but how well the polymer can influence the physical stability of the dispersed drug.

Using a polymer with a high T_g will increase the T_g of its solid dispersions as the API T_g values are generally quite low (here ranging from -45 to 85 °C). Solid dispersions with high T_g values are generally thought to be more stable than ones with a lower T_g .¹⁶ Although some studies have found a strong correlation between increasing polymer T_g and decreasing

crystallization tendency, others have found no such connection or even shown an opposite trend.^{28–31} In this study the three polymers used have a wide range of physicochemical properties, such as T_g , SOL 70 °C, PVP-VA 103 °C, and HPMCAS 120 °C.^{45–47} The results show that generally SOL is the most stabilizing polymer in HME formulations despite having the lowest glass transition temperature. The same does not apply to the spray-dried formulations, where PVP-VA and HPMCAS show better crystallization inhibition properties. However, PVP-VA and HPMCAS were spray-dried at a temperature below their T_g while for SOL this was above its T_g . Whether this affected stability is unclear. It is clear, however, that there are more factors that contribute to stability than just the polymer or solid dispersion T_g .

Stabilizing Interactions. Generally there are four different types of specific interactions that can occur between an API and a polymer, in order of decreasing bond strengths: ionic bonds > hydrogen bonds > dipole–dipole interactions > van der Waals interactions. Ionic bonds are strong attractions between two oppositely charged molecules. A hydrogen bond (H-bond) is an electrostatic interaction between a hydrogen and an electronegative atom (O, N, or F); these can occur both between two identical molecules (API–API) and between different molecules (polymer–API). A dipole–dipole interaction can occur between two polar molecules, when they line up so that the positive part of one molecule is opposite the negative one of the other molecule. The weakest of the interactions is the van der Waals interactions, which are induced dipolar interactions that can occur between any functional groups. Specific interactions between drug and polymer can impact the physical stability of solid dispersions. For instance H-bonds between polymer and drug can hinder crystallization by reducing the amount of drug in the conformation needed for crystallization. H-bonding can also occur between APIs, which can facilitate crystallization. However, if there are more or stronger H-bonds available between API and polymer, or amorphous API–API than between crystalline API–API, this can impede crystallization.^{16,44,48}

In a study of solid dispersions containing vitamin C and several polymers, the influence of H-bonding between polymer and drug on physical stability was considered. As well as H-bonding, the effects of polymer hygroscopicity and T_g were examined. It was found that the formation of strong H-bonds between the polymer and drug in dispersions was the most important factor inhibiting crystallization of vitamin C. The hygroscopicity of the polymer played some part in stability, especially when the solid dispersions were stored at a high RH. No correlation could be found between polymer T_g and physical stability of solid dispersions.²⁸ In a similar study performed by Van den Mooter et al. it was observed that, in the absence of any polymer–drug interaction, polymer T_g was an important stabilizing factor.³⁰

Functional groups that can act as H-bond acceptors or donors are present in all three polymers. H-bond donors include alcohols and carboxylic acids, both present in HPMCAS. Amides (PVP-VA, SOL), esters (SOL, PVP-VA, HPMCAS), and ethers (HPMCAS, SOL) can all act as H-bond acceptors.⁴² Thus, only HPMCAS has H-bond donors while all polymers have H-bond acceptors present. In Table 2 the number of H-bond donors and acceptors are listed for each of the drugs. It can be observed that these numbers vary greatly between the different drugs; H-bond acceptors range from two

to nine while H-bond donors range from zero to four. If the stability of solid dispersions was mostly dependent on the ability of the drug and polymer to make specific bonds such as H-bonds, it would be expected that the drugs with large number of H-bond acceptors would be more stable in HPMCAS than PVP-VA and SOL while drugs with no H-bond donors would not be sufficiently stabilized by PVP-VA and SOL as no interaction could be made. Itraconazole, which has the greatest number of H-bond acceptors, first showed signs of crystallization at 16 and 24 weeks, respectively for HME and spray-dried HPMCAS solid dispersions. However, both aprepitant and ritonavir were very unstable in HME HPMCAS, but both have six H-bond acceptors. Fenofibrate, which has no H-bond donors, was the least stable spray-dried formulation for both SOL and PVP-VA, but the HME SOL was stable through the whole six months of testing. It is therefore clear that there are further properties that contribute to the stability than just the ability of drug and polymer to interact, even though that is probably an important factor.

Water, once absorbed into the formulation, can disrupt any H-bonding between the drug and polymer. Furthermore, water can act as a plasticizing agent and increase molecular mobility.^{49,50} Thus, even if a solid dispersion formulation started out stable with abundance of stabilizing H-bonds, at accelerated conditions these might be removed by water and thus cause instability and crystallization. In most cases for SOL and PVP-VA, crystallization was not observed while the spray-dried formulations were still a dispersible powder but only once they had transformed into a solid glass, which indicates the presence of water. PVP-VA is a very hydrophilic polymer and thus quite hygroscopic, absorbing 10% water at 50% RH. Relative to PVP-VA, HPMCAS and SOL are less hygroscopic, taking up 6–7% water at 75% RH and 10% water at 70% RH, respectively.⁵¹ The effect differences in hygroscopicity have on the stability of the formulations can be seen clearly in the case of the spray-dried formulations above, especially when comparing formulations with PVP-VA and HPMCAS. PVP-VA spray-dried formulations form a glass after a while when exposed to very high humidity, 75% RH. The powder seems to melt or dissolve into a glass, and simultaneously crystals start forming. This transformation of powder into glass did not occur for the HPMCAS spray-dried formulations, even after one year at high humidity. It was deduced that this transformation was mainly due to the hygroscopicity of the PVP-VA and SOL polymer, as it was not seen at less humid conditions (data not shown). As water negatively affects the stability of the dispersion, this transformation can be regarded as a step toward crystallization. Despite the transformation of spray-dried formulations due to uptake of water for PVP-VA formulations, they were still, overall, more stable than the corresponding HME formulations.

Processing Method. Janssens and co-workers studied the influence of processing method on the stability of an itraconazole–Eudragit (acrylic polymers often used in solid dispersion formulations) solid dispersion.⁵² Comparing solid dispersions produced with three different methods, spray-drying, film-casting, and hot melt extrusion, the maximum drug loading that could be stabilized by Eudragit was 27.5% for spray-drying and 15% for each of the other two methods. This shows that the processing method can have significant effects on the stabilization of solid dispersions.⁵²

A comparison of HME and spray-drying with regard to interaction, drug distribution, and homogeneity indicated a lower achievable drug loading by HME than spray-drying.

However, HME formulations were more homogeneous and stable and showed stronger interactions than the spray-dried examples.^{53,54}

Some effects on stability are due to the processing method. In some cases a reason might be quite clear, such as aprepitant being the least stable drug in HME formulations. Aprepitant has a very high melting point, 255 °C, and was extruded at a low temperature (160 °C). Other effects of the processing method are not as clear. Only in four cases was the time of crystallization onset the same for the two methods in two similar formulations: these are SOL–itraconazole, SOL–estradiol, PVP-VA–itraconazole, and PVP-VA–felodipine. In all other cases the onset of crystallization time differed between the two methods. The largest difference was observed for SOL–fenofibrate, where the spray-dried formulation showed signs of crystals after 8 weeks while no crystals were observed in the HME until after one year.

When comparing the two processing methods, it can be observed that, overall, for SOL, HME seems to produce more stable solid dispersions than spray-dried, while the opposite appears to be the case for both PVP-VA and HPMCAS. This indicates that the processing method has a profound effect on the solid dispersion stability. It is unlikely that specific bonds would only be present using one method, not the other; therefore relative stability must be directly linked with attributes of the methods. These might include, for example, residual solvents that can destabilize the solid dispersion, in the case of spray-dried formulations. Different compounds can retain varying amounts of solvent, which might in some cases be responsible for the observed difference in stability.

MLR Models. All six models (Table 4) contain three physicochemical parameters, but no two models contain the same three, showing the complexity of the stability of solid dispersions. However, some general rules can be deduced from the six models. The following are correlated with increased stability of the solid dispersion.

1. Increased T_g of the dispersion (measured or predicted)
2. Increased molecular flexibility of the API
3. Decreased number of H-bond donors (or fluorine/nitrogen atoms)

The first rule is in good agreement with previous studies. As stated above, many studies have considered the correlation between T_g and solid dispersion stability.⁵⁵ This often relates to choosing a high T_g polymer in order to obtain a solid dispersion with a high T_g . Moreover, storing the solid dispersion 50 °C below the T_g of the solid dispersion reduces the molecular mobility to a minimum, thus increasing the stability (reviewed in ref 56). Thus, for storing the solid dispersion at 40 °C, a solid dispersion with a T_g of at least 90 °C would be preferable. Here, 60% of the solid dispersions tested had a T_g at least 50 °C above the storage temperature.

The second rule indicates that, the more flexible API molecules are, the less likely they are to adopt the correct conformation for crystallization. In the models the parameters relating to flexibility are aromatic atom count, rotatable bonds, chiral atoms, and ring count. The parameters hindering flexibility, aromatic atom count and chiral atoms, were negatively correlated with stability while the opposite was observed for rotatable bonds, which increases flexibility. Ring count was positively correlated although it would be expected to be negative, however, this parameter only occurred in the model with the lowest R^2_{adj} . Aromatic rings can interact

strongly through van der Waals interactions and π – π stacking, symmetry drives interactions and intermolecular bonding, while ring structures have been shown to cause dense packing structure in crystals, all of which can drive crystallization.¹⁸

A predictive model using a partial least-squares projection to latent structure discriminant analysis (PLS DA) was built based on experimentally determined glass forming ability of 16 drugs. The model showed a negative correlation between glass forming ability and number of benzene rings, but a positive correlation to branching of the carbon skeleton, molecular symmetry, and distribution of electronegative atoms in the API.⁵⁷ Another study found that APIs with high molecular weight and flexible structures were more likely to be glass formers than small rigid molecules.⁵⁸ Several models using calculated, predicted, and measured parameters have been built to predict the amorphous stability of APIs. In models containing rotatable bonds these were positively correlated with stability while aliphatic rings were negatively correlated.²³ These studies only examined the API alone and not in a solid dispersion as here; moreover two of them only look at glass-forming ability, not stability. However, they show that flexibility and structural complexity of the API is strongly connected with its crystallization tendency, which was highlighted by four flexibility/complexity (nChir) parameters in the models above. It can therefore be concluded that, with regard to flexibility of the API, the same applies to glass forming ability of an API on its own and the stability of the amorphous form in a solid dispersion.

The first two rules are intuitively expected whereas the third one is less so. H-bonds are an important factor to stabilize solid dispersion as discussed before. Several studies have found hydrogen bonding to be important for the glass forming ability or stability of glasses.^{22,23,59} Furthermore, a positive correlation was found between well-distributed electronegative atoms and glass forming ability, which may be due to the H-bonding ability of such atoms.^{18,57} The atomic mass weighted third-order R autocorrelation index ($R3m$) molecular descriptor has been found to be the most significant predictor of miscibility in PVP-VA solid dispersion. This was based on experimental data from 12 APIs. The $R3m$ is associated with electronegative atoms and their positions in the molecule.⁶⁰ None of these studies considered the stability of solid dispersions in the context of H-bonding. Here, however, H-bond donors and acceptors, as well as number of nitrogen atoms, are shown to be negatively correlated with stability, in all cases but for N count in the Ps model. For the three polymers studied here only HPMCAS has any H-bond donors, but all have H-bond acceptors. However, even if there are H-bond acceptors available in the carrier polymer, that does not necessarily mean that API–polymer H-bonds will actually be formed. Instead these H-bond donors/acceptors in the APIs could lead to API–API bonding or API–water H-bonding, both of which lead to decreased stability and more rapid crystallization.

Our proposed rules are intended not to be absolute but to suggest guidelines, and, due to the complexity of these systems, exceptions must be expected. E.g., even though increased T_g is correlated with increased stability of SOL HME, solid dispersions were the most stable of the HME formulations overall, despite SOL having the lowest T_g out of the three polymers. Similar examples can be found for the flexibility. If the two extremes are compared, estradiol is the most rigid API in the study with three aliphatic rings, one aromatic ring, and no non-terminal rotatable bonds. Orlistat is the other extreme

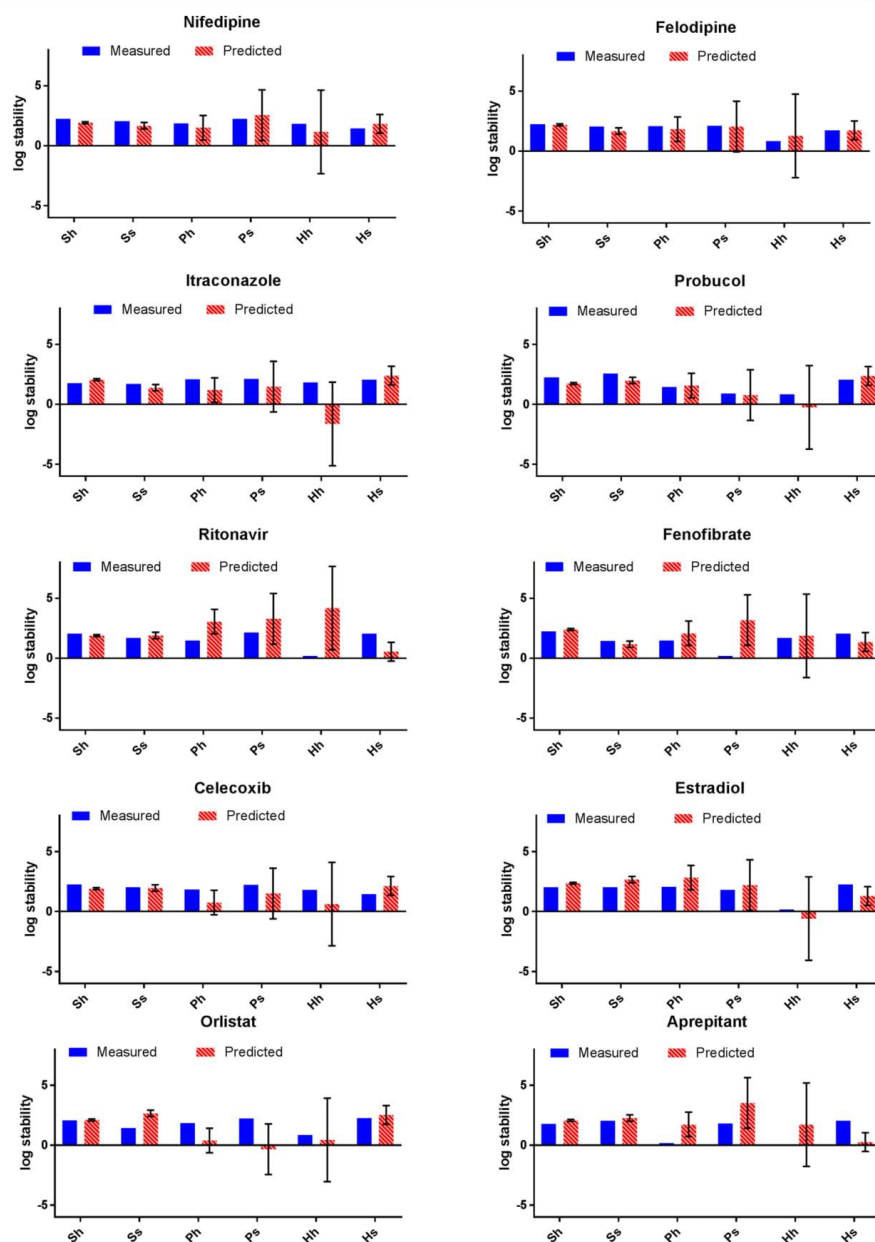


Figure 8. Ranking of formulation combinations of each API and polymer-manufacturing combination. The error bars show the MSE of each model. The predicted ranking is in good agreement with the measured stability. The following abbreviations are used: SH, SOL HME; SS, SOL spray-dried; PH, PVP-VA HME; PS, PVP-VA spray-dried; HH, HPMCAS HME; and HS, HPMCAS spray-dried.

with only one aliphatic ring, no aromatic rings, and 23 rotatable bonds. If solid dispersion stability was only dependent on API

flexibility, orlistat would be expected to be much more stable than estradiol in all formulations. However, this is not the case:

orlistat is more stable in two formulations, PVP-VA spray-dried (52 weeks vs 26 weeks) and HPMCAS HME (4 weeks vs 1 week). In two instances the stability is the same for the two drugs in comparable formulations, SOL HME and HPMCAS spray-dried, while in the latter two formulations estradiol is more stable than orlistat.

The MSE ranges from minimal in Sh model to very large in the PVP-VA models in particular. If the predicted values are compared to the measured ones, they are overall mostly in good agreement. However, especially for the PVP-VA and Hh models there are a few APIs that are very poorly predicted, such as ritonavir in both Ph and Hh or fenofibrate in Ps. These, during the LOOCV process, were predicted using models that varied from the main models. The LOOCV models for these drugs used parameters for which the APIs had extreme values, differing greatly from the rest, such as for ritonavir, both Ph and Hh models contained tPSA; in both cases ritonavir has the highest value by far (145) compared to most others (<100). This difference in chosen models in LOOCV is mostly due to the relatively small data set; with more data the models are more robust and less likely to be as affected by leaving out a single value.

The purpose of these models is to indicate which combination of processing method and polymer carrier is most likely to give a stable solid dispersion. Thus, the models are used to rank the stability of the formulations so that the best combination can be found. Figure 8 shows the ranking of formulation combinations.

When comparing the models using one, two, and three covariates, the following can be observed: as expected the R^2_{adj} falls when number of covariates is decreased. However, when looking at the MSE, this decreases in four cases out of six. The lower MSE might suggest that the higher number of covariates is overfitting the model. In order to test the models against overfitting an external validation is needed.

The external validation shows that the models are nevertheless able to correctly predict the ranking of formulations containing a novel drug. Moreover the predicted stability was in very good agreement with the measured stability, thus showing that, despite a relatively small data set, in terms of statistics, the models can be applied on real data to give a true ranking of formulations, which is promising as a support tool in solid dispersion formulation development.

CONCLUSIONS

60 solid dispersions were produced successfully using ten APIs, three polymers, and two processing methods. An accelerated stability study has shown significant differences in time to crystallization dependent on both choice of polymers and processing method. This extensive experimental data set provides new insights into the complexity of the stability of solid dispersion systems. There are, as previously described, many factors that influence their stability. However, there are some conclusions that can be drawn, such as that HPMCAS and PVP-VA are more likely to give a stable formulation using spray-drying, while HME seems to be more favorable for SOL solid dispersion formulations.

Six predictive statistical models were built, which show the potential of using such models in formulation development. Furthermore, none of the models are identical, which indicates the complexity of these systems. Three rules that could guide formulation development can be deduced. Increased stability is correlated with increased T_g of solid dispersion, as are

decreased number of H-bond donors and increased molecular flexibility of the API. The LOOCV showed good predictability, which was confirmed in an external validation of three formulations.

Future studies are planned to add data to the models to further increase their robustness and possibly to use as an external validation set.

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Notes

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