

Investigation of molecular interactions between active pharmaceutical substances and polymers in amorphous solid dispersions

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

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Düsseldorf, Oktober 2026

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Gedruckt mit der Genehmigung der Mathematisch-Naturwissenschaftlichen
Fakultät der Heinrich-Heine-Universität Düsseldorf

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Tag der mündlichen Prüfung: 09. Oktober 2026

"Today of all days, it is brought home to me: It is no bad thing to celebrate a simple life."

- J.R.R. Tolkien

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List of abbreviations

¹ H-NMR	<i>proton nuclear magnetic resonance</i>
API	<i>active pharmaceutical ingredient</i>
ASD	<i>amorphous solid dispersion</i>
AUC	<i>area under the curve</i>
BCS	<i>biopharmaceutical classification system</i>
BSA	<i>bovine serum albumin</i>
CED	<i>cohesive energy density</i>
CIN	<i>cinnarizine</i>
DFT	<i>density functional theory</i>
DSC	<i>differential scanning calorimetry</i>
DVS	<i>dynamic vapour sorption</i>
EPO	<i>basic butylated methacrylate copolymer</i>
FaSSIF	<i>fasted state simulated intestinal fluid</i>
FTIR	<i>Fourier-transform infrared spectroscopy</i>
HME	<i>hot melt extrusion</i>
HPLC	<i>high-performance liquid chromatography</i>
HPMC	<i>hypromellose</i>
HPMCAS	<i>hypromellose acetate succinate</i>
K30	<i>povidone</i>
K _D	<i>dissociation constant</i>
LAF	<i>laminar airflow box</i>
MAE-A	<i>methacrylic acid – ethyl acrylate copolymer (1:1) Type A</i>
MAE-B	<i>methacrylic acid – ethyl acrylate copolymer (1:1) Type B (partially neutralised)</i>
MWCO	<i>molecular weight cut-off</i>
OPLS	<i>optimised potentials for liquid simulations</i>
PAA	<i>polyacrylic acid</i>
PEG	<i>polyethylene glycol 6,000</i>

Ph. Eur.	<i>European Pharmacopoeia</i>
pK _a	<i>acid dissociation constant</i>
PSSA	<i>polystyrene sulfonic acid</i>
PVAP	<i>polyvinyl acetate phthalate</i>
r.h.	<i>relative humidity</i>
SD	<i>spray drying</i>
SOLU	<i>polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer</i>
ssNMR	<i>solid state nuclear magnetic resonance</i>
TFAM	<i>tangential flow absorption model</i>
T _g	<i>glass transition temperature</i>
TGA	<i>thermogravimetric analysis</i>
T _m	<i>melting temperature</i>
TMP	<i>trimethoprim</i>
TMS	<i>tetramethylsilane</i>
T _{s,mix}	<i>solubility temperature of the drug</i>
USP	<i>United States Pharmacopoeia</i>
VA64	<i>copovidone</i>
VCM	<i>vacuum compression molding</i>
XRPD	<i>X-ray powder diffraction</i>
χ	<i>Flory-Huggins interaction parameter</i>

Publications and contributions

Prepared and submitted manuscripts

S. Schmidt, J. Breitzkreutz, H.A. Schiffter, *On the influence of polymers in stabilising and enhancing the performance of cinnarizine amorphous solid dispersions*, Target Journal: Next Research, 2026.

S. Schmidt, K. Brachmann, L. Esser, J. Duong, J. Breitzkreutz, H.A. Schiffter, *Molecular modelling and experimental investigations into the process-related embedding and dissolution profiles of trimethoprim amorphous solid dispersions*, Target Journal: Journal of Drug Delivery Science and Technology, 2026.

Poster presentations

Parts of this doctoral thesis have been presented in poster presentations:

S. Schmidt, H. A. Schiffter; 2023, *Amorphous solid dispersions of cinnarizine – the influence of polymer type and manufacturing method on drug solubility, partition coefficient and stability*, AAPS PharmSci 360, Orlando, US.

S. Schmidt, H. A. Schiffter; 2023, *Investigation of molecular interactions between active pharmaceutical substances and polymer excipients using 1H-NMR*, AAPS PharmSci 360, Orlando, US.

1 Theoretical background / introduction

1.1 Poorly soluble drugs

Due to its convenience, patient compliance, and relatively inexpensive manufacture, the oral route has historically been and still is the predominant route of drug administration. To effectively deliver a drug via the oral route, the drug substance must be absorbed in sufficient quantity to exert a therapeutic effect. The oral absorption of drugs depends on multiple factors, primarily on aqueous solubility and permeation through membranes. Poor aqueous solubility is a significant point of concern given its limitation on dissolution and hence, the limits on absorption. According to the European Pharmacopoeia (Ph.Eur.) and the United States Pharmacopoeia (USP), the solubility of a drug substance can be classified as shown in Table 1.1. It is estimated that >40% of marketed drug substances, and up to 90% of molecules in development have low water solubility [1–4]. This is largely a result of processes involved in modern drug discovery as high-throughput screenings and combinatorial chemistry, which often produce more lipophilic and higher molecular weight weakly basic molecules. Larger and more hydrophobic molecules often show higher activity in biochemical assays and in molecular modelling by fitting into binding pockets of proteins and receptors, and possess enhanced membrane permeability [5–7]. During lead optimisation, lipophilicity is further increased through chemical modification such as halogenation, addition of alkyl- or aryl-substituents, and reduction of polar functional groups, also in the form of prodrugs. Additionally, the metabolic stability can be increased by these strategies [8,9].

Table 1.1 Solubilities according to the European Pharmacopoeia and the United States Pharmacopoeia.

Description	Part of solvent required per part of solute	Solubility in mg/mL
Very soluble	Less than 1	> 1,000
Freely soluble	1 – 10	100 – 1,000
Soluble	10 – 30	33 – 100
Sparingly soluble	30 – 100	10 – 33
Slightly soluble	100 – 1,000	1 – 10
Very slightly soluble	1,000 – 10,000	0.1 – 1
Practically insoluble	10,000 and more	< 0.1

Another way is to classify not only solubility but also permeation using the biopharmaceutical classification system (BCS). Four classes exist based on both factors influencing bioavailability:

Class 1: High solubility, high permeability

Class 2: Low solubility, high permeability

Class 3: High solubility, low permeability

Class 4: Low solubility, low permeability

Here, “high solubility” is given if the highest single therapeutic dose is completely soluble in up to 250 mL of aqueous media at pH 1.2 – 6.8 at 37 °C, per definition. If more than 250 mL are required to completely dissolve a single dose, the compound has a low solubility. Permeability is assessed based on human pharmacokinetic studies or on Caco-2 cell assays, with “high permeability” meaning an absolute bioavailability of at least 85% [10].

Whilst most drugs that are available as tablet formulations belong to BCS class 1, an increasing number of drugs can be classified in either BCS class 2 or 4 [11,12]. Drugs in these classes represent the greatest challenge for oral drug development. For BCS class 2 substances, the rate-limiting step is dissolution. BCS class 4 compounds are limited by both dissolution and membrane permeability. A high proportion of the affected molecules are weakly basic compounds with a highly pH-dependent solubility. These molecules dissolve to a high degree in the acidic gastric environment but precipitate upon transfer to the intestinal tract, the main site of absorption. The pharmaceutical industry is continually exploring innovative strategies to improve the bioavailability of drugs with poor water solubility [1,2,13].

One option to overcome poor drug solubility in aqueous media is to formulate the respective compounds as an amorphous solid dispersion (ASD) [1,14–17]. In an ASD, the drug is molecularly dispersed in an amorphous state inside a polymeric matrix. The principle and function of ASDs is described in detail in chapter 1.2. Besides ASDs, a variety of other formulation strategies has been developed to address poor aqueous solubility, for example salt formation, micro- or nanosizing of drug particles, co-crystals, lipid-based formulations as self-emulsifying drug delivery systems or lipid suspensions, and cyclodextrin inclusion complexes. Among these, ASDs were established as the most widely used and most promising option for delivering poorly soluble drugs. Between 2012 and 2023, 48 ASD drug products were approved by the U.S. Food and Drug Administration with 36 different drug substances in 10 therapeutic areas [3].

1.2 Amorphous solid dispersions

1.2.1 Principle

ASDs work by converting crystalline drugs into their amorphous form and dispersing them within a polymer matrix, which increases the drugs' apparent solubility and dissolution rate, ultimately enhancing their bioavailability [18,19]. The amorphous form is characterised by higher internal energy, the Gibbs free energy, and a lack of long-range molecular order, enabling higher solubility compared to its crystalline form [20]. The main goal of formulating a drug as an ASD is to enhance its dissolution rate and, subsequently, its bioavailability. ASDs can improve the dissolution of a drug by forming a supersaturated solution [21,22]. However, this supersaturation is frequently only kinetically stable, and in consequence prone to precipitation [23]. Rate and extent of drug release from an ASD are affected by several factors, including drug loading, polymer selection, and molecular interactions between the drug and the polymer [24,25]. The polymers used in ASDs can

be crystallisation inhibitors preserving the supersaturated state, assisting sustained drug release and stabilising the system during storage [26–29].

Though named amorphous solid dispersion, the aim is to dissolve the amorphous drug in the polymeric carrier. If the drug load does not exceed the solubility of the drug in the polymer, the system will be thermodynamically stable [30]. As the solubility is often not high enough to achieve drug loads of adequate concentrations that are completely dissolved, the systems typically are supersaturated in the solid state and therefore prone to crystallisation. Perfectly mixed and homogeneous systems are referred to as “glass solutions”, whilst “glass suspensions” describe systems with supersaturated phases, or drug-rich amorphous phases and polymer-rich amorphous phases. If a crystalline substance is used as the carrier, the resulting systems can be classified as “substitutional solid solution” or “interstitial solid solution”, depending on the molecule size of the solute and therefore its arrangement within the carrier lattice [31]. A special case is the “eutectic mixture”, where both solute and carrier crystallise from a mixture melt at the same temperature, but only at a specific composition ratio [31,32].

The phenomenon of supersaturation in dissolution testing is known as the "spring" effect in dissolution studies, which occurs when the thermodynamic solubility of the drug is exceeded. It has been demonstrated that this state can be reached by the amorphous compound alone. However, in the absence of polymer stabilisation, the concentration of dissolved drug molecules will decrease drastically within a short period, eventually dropping to the thermodynamic solubility. Certain polymers as hypromellose acetate succinate, polyethylene glycol or povidone have shown to stabilise the supersaturated state through diverse mechanisms, for example by forming specific molecular or hydrophobic interactions [26,33]. By this stabilisation, the solubility drop is decelerated and the time frame with increased drug concentration is prolonged. This is especially important as only dissolved molecules can be absorbed via the intestinal membrane. The so-called "parachute" effect can therefore be utilised to achieve a higher bioavailability (see Figure 1.1).

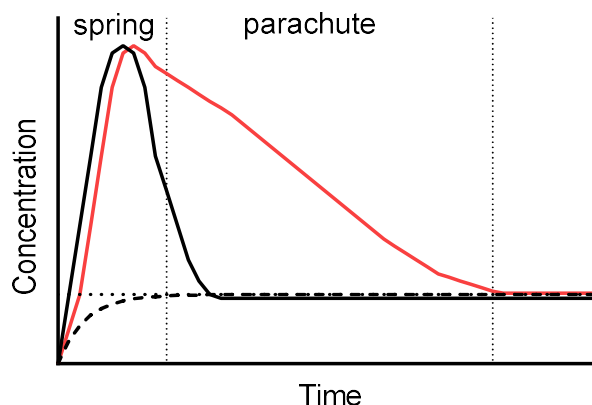


Figure 1.1 Graphical representation of drug concentration-time-curves for a crystalline drug (dashed black line), an amorphous drug (solid black line) and a drug formulated as an ASD (red line). The dotted line shows the thermodynamic solubility of the drug. Vertical lines divide the curves into the spring area (amorphous drug and ASD) and the parachute area (ASD).

In amorphous solid dispersions, the dissolution process is affected by multiple intermediate states and colloidal states, beyond the traditional dissolution process of an active pharmaceutical ingredient (API) or a free soluble crystalline formulation. The states include drug-rich particles containing API or API-polymer mixtures, micelles, or a suspension of crystals [18]. These are all colloidal systems that are not readily available for absorption via the intestinal membrane. Dissolution may create a supersaturated solution, but only up to the amorphous solubility limit and may also lead to liquid-liquid or glass-liquid phase separation, which can be combined to the term amorphous-liquid phase separation [18,19].

The type of the polymer carrier can have implications concerning the dissolution process, as the polymer may restrict dissolution in case it swells and forms a gel matrix through which the drug must diffuse, essentially acting as a depot. Since the typical polymers used in pharmaceutical ASDs are not absorbed in the small intestine due to their larger molecular weight, the only component that is removed from the system is the API, although it is possible for dissolved polymer molecules to spatially separate from the API via convective transport in intestinal fluids.

Depending on the dissolution kinetics, two extremes are possible: First, there can be a high rate of dissolution of the API resulting in fast recrystallisation from solution, as the dissolved polymer concentration is insufficient to stabilise the supersaturated state. Second, if the polymer dissolves faster than the API, the residual drug in the solid formulation can crystallise as the stabilisation potential of the polymer is missing. Though the solid amorphous form of the API is no longer stabilised after polymer dissolution, pre-dissolved polymer molecules help to prevent recrystallisation of dissolved API molecules upon supersaturation in the fluids. The ideal scenario occurs when the rate of dissolution of API and polymer is congruent and a stable fast dissolution of drug-rich particles occurs from the surrounding polymer matrix, with the drug-rich particles having an initial size of 50 – 500 nm [34–36]. It should also be noted that the quality of wetting the surface of the ASD particles initially has a profound influence on dissolution, as shown by Dahlberg et al., due to the potential for molecules at the solid-liquid boundary to rearrange their interactions [37].

Crystallisation inhibition induced by polymers is based on multiple mechanisms, including (i) an increase in drug solubility and in viscosity of the dissolution medium, (ii) a sterical suppression of crystal formation and growth, and (iii) the interaction with the crystal surface, thereby reducing the potential for nucleation and crystal growth [23,38]. Finally, molecular interactions will dictate possible amorphous solubility [39]. It has been demonstrated that the magnitude of API-polymer interactions is proportional to the reduction in amorphous solubility, which is based thermodynamically on the reduction of the chemical potential. In the event of weaker molecular interactions within a drug-polymer system in comparison to a pure system of the active substance alone, the amorphous solubility of the former is known to reach its maximum level. However, this is accompanied by the disadvantage of the system's susceptibility to phase separation in the solid state [27,40].

1.2.2 Manufacturing methods

The way of manufacturing an ASD has a big impact on its physical properties and overall performance [41]. Two common manufacturing methods are hot melt extrusion (HME) and spray drying (SD).

HME involves the melting and blending of a drug together with a polymeric carrier at elevated temperatures and under shear forces to create a homogenous solid dispersion upon cooling [42–44]. Typically, a premixed combination of the active pharmaceutical ingredient and the polymeric carrier is dosed from a controlled feeding unit into a co-rotating twin-screw extruder [19]. In small-scale setups, manual feeding is also an option. Laboratory-scale extruders often have 10 – 20 mm screw diameters and are run at throughputs of only a few grams per minute, which is useful in early stages of development. The cylinder of the extruder has several independently controlled heating zones to achieve a desired thermal profile across the screw length. Temperature settings need to be below the degradation temperature of drug and polymer. The screws have conveying elements on the feed section, then kneading blocks with different stagger angles that provide distributive and dispersive mixing. Mixing intensity also depends on fill volumes and configuration of each barrel section. The length-to-diameter ratio (L/D) often lies between 20:1 and 40:1.

The solid drug-polymer blend is conveyed, melted, and homogenised during the processing step. Any entrapped gases, moisture, or residual solvents are removed by evaporation. The process is monitored by torque values to express the viscosity of the melt, and by temperature to ensure a stable process and reproducible melt properties. The homogeneous melt is passed through a die, typically about 1–3 mm in diameter. Then, the extrudate is cooled rapidly in air or with help of a venting system to preserve the amorphous state. The cooled strands can be post-processed into pellets or can be milled followed by sieving to obtain a uniform size fraction for solid-state characterisation or downstream dosage form manufacture. The versatility of HME is derived from the ability to control feeding, thermal history and shear input. Balancing screw configuration, processing temperatures, and die configuration, the extruder configuration can be optimised to molecularly disperse the drug molecules within the polymeric matrix.

HME is beneficial due to its solvent-free nature, capacity for continuous production, and ability to generate a broad range of dosage forms [19,45]. The careful selection of appropriate polymers, process parameters, and optimisation of extrusion conditions is critical to ensure the stability and effectiveness of the resulting ASD [43,45]. Nevertheless, the elevated temperatures used in HME may present a risk of thermal degradation, making it essential to understand the thermal properties of both the drug and the excipients employed in the formulation [46]. The typical temperatures used in pharmaceutical hot melt extrusion range from 100 °C to 200 °C, depending on the nature of the polymer and the addition of a plasticiser. In marketed ASD products, copovidone is by far the most

prevalent polymer in hot melt extrusion (92%). Besides that, only hypromellose acetate succinate is found in ASD drug products prepared by HME as stabilising polymer [3].

In the spray drying of ASDs, the drug and polymer are dissolved in a suitable solvent, and then rapidly dried by spraying the solution into a hot drying gas stream. This method is better suited for thermally labile compounds and offers good control over particle size and shape [47,48]. Spray drying is the preparation method that is most often used in commercial ASD preparation: 54% of marketed ASD products are manufactured by spray drying, whereas only 35% of the products are made using hot melt extrusion, as stated in an article by Moseson et al. [3]. Still, potential issues include residual solvent in the final product, safety concerns, and more complicated processing steps.

The process starts with dissolving the drug and one or more polymeric carriers in a volatile organic solvent or solvent mixture. This feed solution is delivered to the spray dryer, where it is atomised into small droplets using a two fluid nozzle, pressure nozzle, or rotary atomiser. The droplets are injected into a drying chamber where the heated process gas, usually nitrogen, is passed in a co-current or counter-current direction. Inlet temperatures are chosen to achieve fast solvent evaporation without exceeding the degradation temperatures of the drug and the polymeric carriers, often in a range of 70 – 110 °C. Outlet temperatures are usually lower and indicate the extent of drying. As the solvent evaporates from the rapidly shrinking droplets, solid particles are formed with the drug being molecularly dispersed in the polymeric carrier.

The dried solids are separated from the drying gas via a cyclone and then collected for downstream processing. Spray-dried powders may undergo a further drying step, usually under vacuum to eliminate any residual solvent trapped in the powders. Spray-dried powders are typically in the size range of < 1 µm to 100 µm [49]. The speed of solvent loss at the droplet surface can create a dense or hollow particle shell, affecting dispersibility and dissolution characteristics. Polymers used in spray drying of ASDs also include copovidone, but hypromellose acetate succinate, hypromellose and other cellulose derivatives are more strongly represented [3].

Both methods, HME and spray drying, can produce effective ASDs but they do not necessarily result in the same product [50–53]. Differences in processing, especially factors such as temperature, solvent evaporation rate, and mixing, can influence how well the drug and polymer interact on a molecular level. These interactions (such as hydrogen bonding or π - π interactions) are key to keeping the drug stable in the amorphous form.

Another option to form the amorphous state is by mechanical energy input in milling processes. The drug substance in its crystalline form can be milled together with one or more polymers to transform it into the amorphous form and embed it into the polymeric carrier without the need for organic solvents or elevated temperatures. To reduce the thermal stress produced by the milling process, cryomilling can be a viable option. Both methods are limited in terms of their efficiency and reproducibility, which manifests in the fact that there is no marketed ASD product produced by

milling or cryomilling. Still, it might be a useful approach for small-scale screening. The same is true for lyophilisation, which is also not suitable for producing ASDs at industrial scale, as it is energy intensive and slow compared to other drying techniques. Typically, freeze-drying is performed with aqueous solutions, which also limits the applicability for poorly aqueous soluble drugs, except for cases where the dissolved polymer increases the solubility of the active significantly. Other methods include spin coating, 3D printing, and supercritical fluid processing. The KinetiSol method combines the mechanical input in form of frictional and shear forces from milling with the elevated temperatures from melt extrusion to prepare ASDs in less than a minute, even with thermally labile drug compounds. A number of review articles provide a comprehensive overview the various technologies [54–56]. Small-scale approaches such as vacuum compression molding or other forms of melt quenching in fixed dies are more commonly used for formulation screening and are not suitable for large quantities.

1.2.3 Long-term physical stability

One of the primary challenges related to ASDs is their long-term chemical and physical stability [36,57]. Amorphous forms are susceptible to recrystallisation over time, particularly under stress conditions such as enhanced temperature and humidity [58,59]. The free energy difference between amorphous and crystalline form provides the driving force for recrystallisation and can be described by Eq. (1.1) [60,61].

$$\Delta G_{a-c} = -R \cdot T \cdot \ln \left(\frac{a_a}{a_c} \right) \quad (1.1)$$

ΔG_{a-c} = Gibbs free energy difference between amorphous and crystalline phase; R = universal gas constant; T = temperature; a_a = activity of saturated solution of the amorphous phase; a_c = activity of the dissolved crystalline phase

The physical stability of ASDs is essential, as recrystallisation of the drug can result in a substantial decrease in solubility and dissolution rate. Predictive models, such as the Gordon-Taylor model and the implementation of the Flory-Huggins interaction parameter, aid in estimating the likelihood of phase separation and recrystallisation in ASDs [40,62–64]. Stability may be enhanced by choosing polymers that interact favourably with the drug, thus raising the glass transition temperature (T_g) and providing a kinetic barrier to crystallisation [65,66]. With a higher glass transition temperature, the molecular mobility in the system is decreased at typical storage conditions, which prolongs the timeframe in which crystallisation inhibition is possible. Miscibility between drug and polymer is also crucial, as phase separation and the formation of drug-rich domains can induce the crystallisation process as the system is locally supersaturated and thus destabilised.

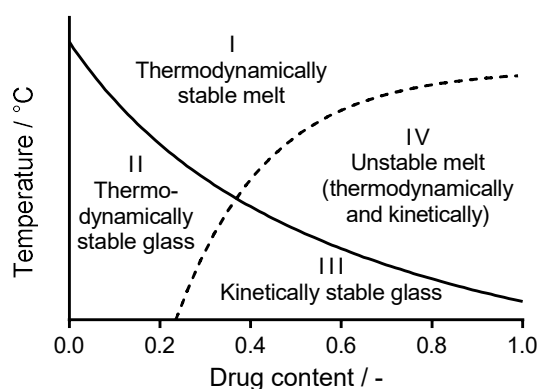


Figure 1.2 Schematic phase diagram of a binary amorphous solid dispersion with four phases and their labelling. The solid line represents the glass transition temperature, and the dashed line represents the solubility temperature of the system.

Phase diagrams are essential instruments for comprehending the thermodynamic and kinetic behaviour of drug-polymer systems [65,67,68]. They offer insights regarding miscibility, solubility, and the stability of the amorphous phase, enabling formulators to forecast the conditions under which a drug will maintain its amorphous state or experience phase separation [68]. By plotting the temperatures for both the glass transition and the solubility against the drug load, a diagram with four different areas results as shown in Figure 1.2 [30,69]. This presentation of drug solubility and glass transition temperature was first published by Yu et al. [70,71]. Whilst phases I and II are thermodynamically stable, phase III can only be kinetically stabilised and phase IV represents an unstable system due to the high molecular mobility at temperatures above the T_g [72,73]. ASDs typically fall within phase III.

1.2.4 Analytical characterisation

ASD characterisation can be divided in (i) solid state characterisation and (ii) dissolution or solubility studies. The solid-state characterisation focuses on the physical attributes with the most important being the presence and degree of crystallinity together with phase homogeneity. Techniques such as Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared Spectroscopy (FTIR), and Solid State Nuclear Magnetic Resonance (ssNMR) are widely employed to investigate the thermal behaviour and possible interactions at the molecular level [14,62,74,75].

In DSC, the heat flow into or out of a sample is measured as a function of temperature or time relative to a reference. It provides information on the thermal transitions of a sample such as melting, glass transition, crystallisation, and decomposition. The ability to detect multiple T_g s enables the visualisation of phase separations. Conventional DSC typically measures the total heat flow signal dH/dt .

$$\frac{dH}{dt} = c_p \frac{dT}{dt} + f(T, t) \quad (1.2)$$

dH/dt = heat flow signal; c_p = heat capacity; dT/dt = heating rate; $f(T, t)$ = kinetic heat flow

The first summand in Eq. (1.2) is dependent on the heat capacity and proportional to the heating rate, meaning it can be influenced by the experimental conditions. The second term, the kinetic heat flow, is the process heat flow which can depend on the temperature, but not on the heating rate. As a result, the conventional DSC measurement yields a total signal for heat flow that is a combination of the reversible component (heat capacity related) and the non-reversible component (kinetically active). Modulated DSC (mDSC) is a more sophisticated version of DSC that applies a sinusoidal modulation of temperature on top of a linear rate of heating. The reversing heat flow captures only events that can reach equilibrium, such as glass transitions. The non-reversing heat flow captures the kinetic portion only, such as enthalpic relaxation, crystallisation, or melting. The primary advantage of mDSC over conventional DSC is that it separates the overlapping thermal events and can characterise the material behaviour more accurately [74].

In the context of ASDs, DSC is a key tool to assess the physical state of a drug and polymer, their miscibility, and potential interactions. The observation of a single glass transition temperature in the thermograms is indicative of molecular-level mixing and homogeneous dispersion of the drug within the matrix [76–79]. The T_g can shift relative to that of the active substance, reflecting specific drug-polymer interactions or plasticising effects [66]. This type of evaluation is also used to construct phase diagrams [15,65,80,81]. In DSC analysis, recrystallisation events can be detected during heating or cooling, providing insights into the physical stability of ASDs, also during long-term storage. The crystallisation and melting enthalpies can be used to calculate crystalline portions within a drug-polymer system as a physical mixture or an ASD.

X-Ray Powder Diffraction (XRPD) is a non-destructive analytical technique to characterise materials based on their crystalline structure. When a powdered sample is irradiated with monochromatic X-rays, the atoms in the crystal lattice scatter the attenuated beam, which is predictable by Bragg's law as shown in Eq. (1.3). That happens when, under certain conditions, the scattered X-rays constructively interfere, producing a discrete number of diffraction peaks at defined 2θ -values.

$$n \cdot \lambda = 2d \cdot \sin\theta \quad (1.3)$$

n = diffraction order; λ = wavelength of X-ray; d = distance in crystal / “grating constant”; θ = angle between X-ray beam and grating plane (“Bragg angle”)

The positions and intensities of the peaks delineate a unique pattern or fingerprint of the crystalline phases in the material. XRPD is a widely used analytical technique to determine the crystallographic state of matter or in case of amorphous solid dispersions if it is completely amorphous or not. [33]. In addition, XRPD is an effective way to confirm polymorphic modification. If a sample seems to lack sharply defined diffraction peaks and instead exhibits a broad halo, it can be concluded that long-range order is lacking. If diffraction peaks appear after testing the sample, evidence for an organised system within the powdered sample is found and crystal domains are

detected. The reliability on the results is highly dependent on a correct sample preparation. In general, thermal analysis as DSC is more sensitive in detecting crystalline domains by their melting signals opposed to XRPD [41], where crystalline portions of ~10% are required for detection [82,83]. The detection limit can be decreased by adjusting the scan time to prolonged analysis.

Besides classifying an amorphous solid dispersion as crystalline or amorphous, also the degree of crystallinity can be determined [84]. The crystallisation pathway by recrystallisation and transformation into another polymorphic form, as well as its inhibition by the polymeric carrier, can be analysed by differentiating between polymorphic forms of the residual crystalline drug [84]. In literature, a combined DSC-XRPD setup is described by Clout et al. [85] and Askin et al. [86]. Here, an X-ray beam passed through the calorimeter cell and the aluminium pans, recording diffraction patterns in defined time intervals during heating of the pan. During crystal formation upon heating, the development of Bragg peaks can be observed and by this, the formed polymorph can be classified.

Solid-state Nuclear Magnetic Resonance is a powerful spectroscopic tool that can be used to gain knowledge of the local molecular environment of amorphous and crystalline systems [74]. ssNMR is sensitive to short-range interactions and offers an atomic level of understanding of multiple molecular features that will ultimately determine drug-polymer miscibility, molecular mobility, and intermolecular interactions such as hydrogen bonding. Due to ssNMR's non-destructive nature and ability to allow distinction of chemical moieties, ssNMR serves as a complementary method to the use of DSC and XRPD to form a comprehensive picture of solid-state characterisation. While ssNMR is a well-established technique for analysing molecular interactions within ASDs, liquid-state NMR remains a relatively unexplored area in this context [30,74]. Currently, the only known applications of liquid-state NMR in ASD research are qualitative [23]. Although H-NMR is commonly employed in ligand-protein binding assays to quantify interaction parameters [87–90], a comparable quantitative approach for characterising interactions in ASDs has yet to be established.

In addition to DSC, XRPD and NMR techniques, further analytical methods are widely employed to characterise ASDs. Vibrational spectroscopic methods as FTIR and Raman spectroscopy can be used to detect molecular interactions, for example hydrogen bonding between drug and polymer [91,92]. As both molecular interactions and physical stability of the ASDs are highly dependent on their moisture content, dynamic vapour sorption is used to evaluate hygroscopicity and potential humidity-driven phase changes under controlled temperature and humidity conditions. Water-induced phase separation can also be monitored via confocal fluorescence microscopy [93]. Another part of analysis are imaging methods as scanning and transmission electron microscopy as well as atomic force microscopy and micro-computed tomography [94–97]. These methods can elucidate the morphology, phase separation phenomena and polymer coverage at micro- to nanoscales. Hot-stage microscopy allows the direct visualisation of crystal nucleation and growth as well as phase transitions during thermal or humidity stress experiments [98].

The main characteristic of an ASD in solution is its dissolution behaviour. Standard methods such as the USP Apparatus II are widely used. To better mimic the course of the drug in the gastrointestinal tract, setups with pH changes are often applied. More advanced setups, such as the tangential flow absorption model (TFAM) may offer a broader picture [99]. They can assess not only dissolution and supersaturation, but also mimic absorption process over a membrane and therefore removal of API molecules from the site of drug dissolution. Other options are flow-through cells (USP apparatus IV) and biphasic dissolution systems with organic, lipophilic solvents as octanol. In addition, the medium used for dissolution testing is also an important factor, with many studies using simulated gastric or intestinal fluids, and sometimes adding surfactants, to examine the consequence of solubilisation conditions on drug release.

1.3 Molecular interactions

Molecular interactions are the driving forces behind all chemical and biological processes. They govern how atoms and molecules attract, repel, and react with one another. Broadly, these interactions can be divided into polar and non-polar types, depending on the nature of the participating molecules. Each category comprises various specific interaction types, contributing differently to the behaviour and properties of the molecular system (see Table 1.2) [100–104].

Table 1.2: Categories of molecular interactions between two non-covalently bond atoms.

Polar		Non-Polar
At least one charged molecule	Uncharged molecules	
• Ionic bonding / salt bridges • Ion-dipole • Ion-induced dipole forces • Cation-π interactions	Van der Waals forces	
	• Keesom forces (dipole-dipole; special type are H-bonds) • Debye forces (dipole-induced dipole)	London dispersion forces
		• π-π stacking • Hydrophobic effect

1.3.1 Polar interactions

Polar interactions occur in case of an uneven distribution in the electron density within molecules, either by real charges in ions or in overall neutral molecules, where dipoles can be formed. These dipoles can be either permanent, arising from differences in electronegativity between atoms in covalent bonds, or induced, resulting from perturbations in electron clouds by nearby charges.

Ionic bonding occurs when electrons are transferred from one atom to another, resulting in positively and negatively charged ions. The ions can form strong electrostatic interactions with counterions or with charged regions on macromolecules, for example proteins or synthetic polymers. Interactions between ions and permanent dipoles occur for example when dissolving ions in water.

They are crucial to the solvation process of salts in polar solvents. For example, when sodium chloride is dissolved in water, the positive Na^+ -ions interact with the partially negative oxygen atoms in water molecules, while the negative Cl^- -ions interact with the partially positive hydrogen atoms. An additional behaviour can be observed when a dipole is induced by the ion instead of being permanent [105,106].

Cation- π interactions occur between a positively charged group, such as a protonated amine and the delocalised electron cloud of an aromatic ring. Cation- π interactions are particularly relevant in molecular recognition processes, including ligand-receptor binding and protein structure stabilisation [107,108].

There can be interactions between two permanent dipoles (Keesom interactions), for example between molecules of the same kind, and interactions between a permanent and an induced dipole (Debye interactions) [105,106]. In a molecule such as hydrogen chloride, the more electronegative chlorine atom pulls electrons away from the hydrogen atom, creating a partial negative charge on chlorine and a partial positive charge on the hydrogen and thus creating a permanent dipole. The partial charges align favourably with the opposite charges in neighbouring molecules. This leads to an energetically favourable structure. Such interactions, while relatively weak compared to covalent or ionic bonds, significantly influence boiling points, melting points, and solubilities of polar compounds. A special kind of Keesom interactions are hydrogen bonds, where a hydrogen atom, which is covalently bonded to an element with high electronegativity as nitrogen or oxygen, interacts with another nitrogen or oxygen atom. Hydrogen bonds are crucial in determining the secondary and tertiary structures of proteins and other macromolecules as polymers. Due to atom radii and steric hindrance, not all Keesom interactions are equal to hydrogen bonding. With hydrogen bonding, interatomic distances can be shorter than the sum of their van der Waals-radii. Examples for Debye interactions can be found in combinations of permanent dipoles, as hydrogen chloride, with noble gases as argon [109].

1.3.2 Non-polar interactions

π - π stacking refers to the attractive forces between aromatic rings. The directionality of π - π stacking can vary, but it typically occurs in either a face-to-face (sandwich), edge-to-face (T-shaped), or offset parallel (slipped) geometry, with the slipped-parallel arrangement being the most energetically favourable [107,108].

Though van der Waals forces include Keesom, Debye and London dispersion forces, the latter is typically referred to when talking about van der Waals forces. Here, a fluctuating dipole induces another dipole and then interacts with it. An atom with a large number of electrons has a greater associated London dispersion force, as electron fluctuations are more common. In general, London dispersion forces are present in all atoms and molecules regardless of their polarity but are mainly

governing the behaviour of non-polar molecules as hydrocarbons. The strength of an individual interaction is weak compared to polar ones, but due to their widespread presence, they cumulate in large systems with long-chain alkanes and aromatic compounds. Van der Waals forces are proportional to r^{-6} , with r being the distance between the involved atoms, therefore covering a very short distance range compared to electrostatic forces, which are reduced proportionally to r^{-2} [109,110].

Besides the already mentioned types of interactions, there is also the hydrophobic effect influencing the folding of macromolecules in solutions or the arrangement of amphiphilic molecules. Long, non-polar side chains can cluster to exclude hydrophilic solvent molecules as water and thereby reducing the contact area, which yields in a reduction of the disturbance of the water molecule lattice [111,112].

1.3.3 Molecular interactions in ASDs and methods to assess molecular interactions

The stability and effectiveness of ASDs are fundamentally governed by the molecular interactions that occur between the drug and the polymeric carrier [40,57,76]. These interactions, which may encompass salt bridges or ionic bonding, hydrogen bonding, van der Waals forces, π - π stacking, and hydrophobic interactions as water-exclusion, are essential in keeping the drug in its amorphous state and preventing recrystallisation [15,26]. Hydrogen bonding often influences the miscibility and physical stability of the dispersion. Strong hydrogen bonds can prevent crystallisation of the amorphous drug and thereby enhance dissolution rates. This was shown by Wu et al. [73] especially for drugs with low crystallisation tendency, while fast crystallisers could not be easily stabilised by hydrogen bonding with the polymer. Another study showed a similar effect, where hydrogen bonding between drug and polymer disrupted self-associations of indomethacin and thereby enhanced miscibility and stability [113].

Besides hydrogen bonding, nonpolar or neutral interactions, and hydrophobic interactions also play a substantial role in the behaviour of ASDs. These interactions contribute to the miscibility and stability of the system in a comparable manner, meaning by disruption of self-association of the single components and thereby reducing phase separation. This is especially the case for poorly soluble drugs with no or low polarity, which can be formed to larger extents with nonpolar sidechains of uncharged polymers [114].

Still, it is challenging to determine the present interactions qualitative and, even more, on a quantitative level. In other scientific fields such as biochemistry, several methods to quantify the binding capability and bond strength between a ligand and a macromolecule as proteins or DNA exist. These include fluorescence assays and fluorescence anisotropy, isothermal titration calorimetry, surface plasmon resonance, affinity chromatography, microscale thermophoresis and analytical ultracentrifugation, only to name a few. The challenges in transferring these methods to drug-polymer systems as in ASDs are the nonspecific binding as opposed to ligand-protein

complexes, the poor solubility in aqueous media, and the binding of often required markers or labels on the polymer. In the past years, molecular modelling became more popular among biochemists to assess the bonds and interactions *in silico*. This approach is promising for drug-polymer systems as well (see section 1.4 for more details on ASD-relevant applications).

1.4 Molecular modelling

1.4.1 Molecular dynamics / quantum mechanics

Molecular modelling is a computational approach to visualise, simulate, and analyse the structure, dynamics, and interactions of molecules. It includes a set of computational techniques used to represent and predict the behaviour of molecular systems. These methods range from quantum mechanical calculations to classical simulations.

In molecular modelling, systems are constructed by defining the initial molecular geometries, followed by placing them within a simulation environment, such as a periodic cell. Periodic cells can contain either one substance or a mixture of different molecules, as depicted in Figure 1.3. Additionally, appropriate boundary conditions and solvent models need to be defined. Mixed cells allow for the study of solubility, miscibility, and interaction potentials between components, such as drug molecules and polar or non-polar solvents.

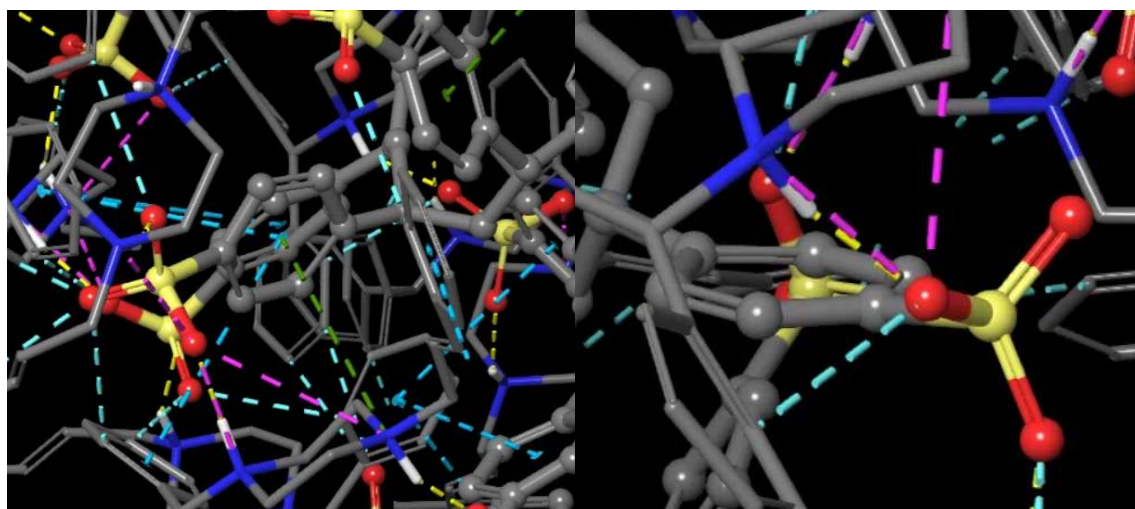


Figure 1.3: Screenshots from the Schrödinger MS Maestro software showing the complex interaction pattern of cinnarizine with polystyrene sulfonic acid, both with charged moieties. Shown interactions are hydrogen bonds (yellow for aliphatic ones, light blue for aromatic ones), salt bridges (pink), π - π stacking (blue), and cation- π interactions (green).

The movement of molecules in these simulations is governed by Newtonian mechanics, where the force on each atom is calculated from a potential energy function. These movements include translations, rotations, vibrations, and larger conformational changes such as protein folding. Molecular folding is influenced by a combination of intramolecular forces (e.g. hydrogen bonding, electrostatics, hydrophobic effects) and steric hindrance, as overlapping van der Waals-radii repel the atoms and thereby set the allowable conformational space [115–117]. During the simulation,

stable configurations with energetically favourable structures are detected. The timeframe covered by simulations is often in the nano- to microseconds range.

The potential energy surface of a molecular system is described by a force field. A force field is a mathematical construction made from equations and parameters to model covalent bonds and non-covalent interactions. It defines how the energy of a molecular system depends on the positions of its atoms. For covalent bonds, stretching, angle bending and dihedral angle torsion are included, while non-bonded interactions include van der Waals and electrostatic forces. The general equation for a force field is shown in Eq. (1.4), where the vector F is the force and U is the potential energy. It can be derived that the force is equal to the negative gradient of the potential energy [117]. It can be distinguished between the electrostatic potential according to Coulomb's law and the Lennard-Jones potential, based on London dispersion forces [118].

$$\vec{F} = -\vec{\nabla}U(\vec{R}) = -\vec{\nabla}(U_{\text{bond}} + U_{\text{angle}} + U_{\text{dihedral}} + U_{\text{electrostatic}} + U_{\text{van der Waals}}) \quad (1.4)$$

F = force; U = potential energy; R = atomic coordinates

The OPLS (optimised potentials for liquid simulations) force field, used in the Desmond simulation engine within Schrödinger's Maestro software, is widely used due to its balance between computational efficiency and accuracy in modelling. OPLS includes parameters that have been optimised to reproduce liquid properties and thermodynamic observations, making it well suited for simulations of solvated molecules and drug-like compounds.

Molecular modelling is applied in ASD research to determine the miscibility behaviour of drugs and polymers, and to visualise potential phase separation [119–121]. Furthermore, thermal properties of the single components or of mixtures can be calculated, for example the glass transition temperature. Studies show that the calculated values are well in line with experimental ones [120,122]. Docking simulations can also be performed to detect specific binding patterns, molecule distances and potential pocket forming. The type of present interactions, which is not accessible for example in DSC studies, can also be determined via modelling [120]. In a larger scale, simulations to calculate the dissolution behaviour of a drug from different polymeric carriers can be applied [14,123]. With machine learning software coupled to modelling tools, predictions of API-polymer-behaviour are possible [122].

1.4.2 pK_a modelling

The acid dissociation constant, pK_a , is a fundamental quantity that describes the tendency of a molecule to donate or accept protons in solution. It provides insight into the molecule's protonation state at a given pH and therefore affects solubility, permeability, and molecular interactions. It defines the pH at which a molecule exists as 50% protonated and 50% deprotonated. This relationship is shown in the Henderson-Hasselbalch equation (Eq (1.5)). $[A^-]$ and $[HA]$ represent the concentrations of the deprotonated and protonated form, respectively.

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad (1.5)$$

With this equation, one can calculate the relative proportions of protonated and deprotonated species at a given pH value [124]. When the pH exceeds the pK_a , the deprotonated form is mainly present (being the charged form for acids and the neutral form for bases). When the pH is lower than the pK_a of a dissolved substance, the protonated form predominates (being the neutral form for acids and the charged form for bases). The pK_a can be determined in aqueous solutions by acid-base titration at the half-equivalence point.

Another approach to determine the pK_a is by *in silico* calculations. Quantum chemical approaches, such as those implemented in Jaguar (a component of Schrödinger's MS Maestro software suite), offer accurate pK_a predictions. Jaguar employs the density functional theory (DFT) to compute the free energies of protonated and deprotonated species [125]. The calculations include a geometry optimisation of both species, energy calculations and an entropic correction factor. Schrödinger states that the determined pK_a -values possess an accuracy of 0.2 – 0.5 pK_a -units for bases and 0.2 – 1.0 pK_a -units for acids.

The protonation state of a molecule influences not only its charge distribution but also its physicochemical properties, including solubility, membrane permeability, and intermolecular interactions. It is well established that the presence of a positive or negative charge on a molecule increases polarity and hydrogen bonding capacity, enhancing solubility in polar solvents such as water. For example, amines are more soluble in water when protonated to form ammonium ions, whereas carboxylic acids are more soluble in their deprotonated, charged carboxylate form.

The change in charge due to protonation or deprotonation also affects molecular interactions in complex systems. In a modelling context, it is therefore crucial to assign the correct protonation state based on the target pH of the system [126]. Polar interactions are supported by charged substances, as ion-dipole and ion-induced dipole as well as strong salt bridges can be formed. However, an increase in charge reduces lipophilicity, which in turn reduces interactions with non-polar molecule.

1.4.3 Solubility parameters

Based on the rule of thumb “like dissolves like”, solubility parameters can be used to assess the “likeness” of molecules and to give hints on potential miscibility or dissolution hurdles. The first and simplest model for this purpose is the Hildebrand solubility parameter δ , based on the square root of the cohesive energy density (CED) of a substance (Eq. (1.6)) and typically reported in $\text{MPa}^{1/2}$ [127]. ΔE_v is the energy of vaporisation of the substance and V_m its molar volume. The energy of vaporisation can be obtained from the heat of vaporisation, ΔH_v , by subtracting the ideal gas constant multiplied by the absolute temperature in Kelvin [128,129].

$$\delta = \sqrt{CED} = \sqrt{\frac{\Delta E_v}{V_m}} = \sqrt{\frac{\Delta H_v - R \cdot T}{V_m}} \quad (1.6)$$

δ = solubility parameter; CED = cohesive energy density; ΔE_v = energy of vaporisation of the substance; V_m = molar volume; ΔH_v = heat of vaporisation; R = ideal gas constant; T = temperature

The more similar the solubility parameters of two substances are, the more likely they are to be miscible, as they possess comparable interaction patterns. Breaking of interactions within one component is compensated for by building new interactions with the second component; as the required and released energies are of comparable magnitude, the overall needed energy is reduced to a minimum [130]. The solubility parameters can be calculated by a group contribution method or determined via intrinsic viscosity measurement, solubility screening, inverse gas chromatography or thermal analysis [129–131]. The approach developed by Hildebrand works best for non-polar and aprotic systems, such as hydrocarbons, and does not account for specific interactions like hydrogen bonding [131].

Hansen, who introduced a three-component model following Eq. (1.7), further developed the calculation [132]. The total solubility parameter δ_t consists of a dispersive (δ_d^2), an electrostatic or permanent dipole (δ_p^2) and a hydrogen bond (δ_h^2) component. These parameters allow for a multidimensional assessment of the solubility behaviour. Two chemical substances are expected to be compatible if all three dimensions are comparable.

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (1.7)$$

δ_t = total solubility parameter; δ_d = dispersive solubility parameter; δ_p = electrostatic solubility parameter; δ_h = hydrogen bond solubility parameter

The dispersive parameters describe non-polar interactions, which result from the atoms themselves and are therefore presents in all types of molecules. δ_p^2 includes the polar cohesive energy resulting from permanent dipoles, and δ_h^2 adds the hydrogen bonding and by this an additional polar interaction type; it is also referred to as electron exchange parameter [132].

Besides studies on the miscibility or dissolution of a chemical substance in a solvent, the approaches can also be used to identify compatibilities of small molecules with macromolecules such as polymers in amorphous solid dispersions [40,133]. Information on the compatibility helps to assess the physical stability of the system in long-term storage and the formation of a homogeneous system in general [134]. Another application includes the assessment of absorption sites in the gastrointestinal tract based on the chemical structures as shown by Obradović et al. [135], and the combination of polymeric detectors for various analytes in electronic nose sensors [128].

2 Aim and objective of the thesis

In spite of the extensive investigations into ASDs, a knowledge gap persists regarding the effects of molecular interactions on both the stability and dissolution performance of these systems. This work intended to address this gap by concentrating on the influence of molecular interactions on the solid state of ASDs after melt quenching, hot melt extrusion, vacuum compression molding and spray drying, and examining the liquid state interactions using ^1H -NMR titration and dissolution experiments. A systematic approach tried to yield a deeper understanding of how different functional groups of the polymers affect the stability and performance of ASDs featuring cinnarizine (CIN) and trimethoprim (TMP) as model drugs, both poorly soluble in water and gastrointestinal fluids [136–138]. Both drug substances are of weakly basic character with nitrogen atoms prone to protonation.

The main aims of the work were

- to manufacture ASDs with various polymers including acidic, neutral, and basic moieties by different manufacturing methods as hot melt extrusion and spray drying with two weakly basic model APIs,
- to characterise the ASDs in their solid state by means of residual crystallinity,
- to perform long-term stability studies on selected formulations at different storage conditions with solid state characterisation at distinct time points,
- to analyse the dissolution behaviour in different dissolution testing equipment: paddle apparatus and tangential flow absorption model,
- to develop a method to compare and rank the interaction potential between drug and polymer in the dissolved state and compare methods to do so in the solid state and
- to apply molecular modelling approaches as molecular dynamic simulations on drug-polymer systems.

3 Materials and methods

3.1 Materials

3.1.1 Active pharmaceutical ingredients

As model active pharmaceutical ingredients cinnarizine (#Z21G093 and #J64586, both Thermo Scientific, USA) and trimethoprim (#DKA679, BLD Pharmatech, China) were used in this work. As determined by DSC, cinnarizine has a glass transition temperature of 8 °C and a melting point of 120 °C. It is classified as BCS class II substance due to its poor solubility of less than 1 µg/mL in water and buffered systems with a pH of > 5.0. The solubility is highly pH dependent with a substantial increase in acidic solutions. In 0.01 M HCl, the solubility increases to 0.8 mg/mL, as determined within this work. Cinnarizine's pharmacological mechanism of action is the regulation of cellular calcium concentrations as a calcium channel blocker and thereby promoting blood vessel dilation. It is used in the treatment of nausea and vomiting as well as for blood circulation disorders [138].

Trimethoprim is also considered as BCS class II substance, though its solubility is higher compared to that of cinnarizine. Trimethoprim has a solubility of 0.5 mg/mL in water, 1.7 mg/mL in phosphate buffer pH 6.8 and 3.7 mg/mL in 0.01 M HCl. The glass transition temperature and melting point are 59 °C and 202 °C, respectively. It is used as an antibiotic, which inhibits the growth of bacteria by attacking their folic acid metabolism. Both cinnarizine and trimethoprim are weakly basic substances with two and four nitrogen atoms in their molecular structures, respectively (Figure 3.1). The nitrogen atoms in cinnarizine are positioned in the piperazine moiety of the drug and no other heteroatoms are present in its structure. For trimethoprim, two of the four nitrogen atoms are primary amines as substituents of the pyrimidine ring. Due to their low basicity, they are not considered as viable protonation sites in later discussions (pK_a values were calculated and discussed in chapter 4.3.16). The oxygen atoms in the ether moieties are also not considered as relevant protonation sites due to their higher electronegativity and tighter held electrons compared to the amine positions.

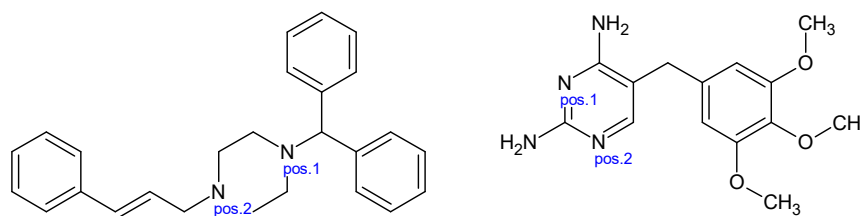


Figure 3.1 Molecular structures of cinnarizine (left) and trimethoprim (right) with marked nitrogen atoms for protonation. The primary amines in trimethoprim are not considered as possible protonation sites due to their low basicity.

3.1.2 Polymers

The polymers used for the preparation of amorphous solid dispersions as well as for testing of solubility and partition influence and NMR analyses are listed in Table 3.1. In the following chapters of this thesis, the polymers are abbreviated as indicated in the second column of Table 3.1. The chemical structure of the mainly used polymers is depicted in Figure 3.2.

Table 3.1 Polymers used in this work.

Substance / trade name	Abbreviation / synonym	Supplier	Batch / lot
Methacrylic acid – ethyl acrylate copolymer (1:1) Type A Eudragit L100-55	MAE-A	Evonik Industries, Germany	#B17040421
Basic butylated methacrylate copolymer Eudragit E PO	EPO	Evonik Industries, Germany	#G170813612
Hypromellose acetate succinate AQOAT HPMCAS-HF	HPMCAS	Shin-Etsu Chemical, Japan	#6033048
Hypromellose Affinisol HPMC HME 15LV	HPMC	DuPont de Nemours, USA	#F293K45018
Copovidone Kollidon VA64	VA64	BASF, Germany	#4396412440
Povidone K-30 Kollidon 30	K30	BASF, Germany	#2569762440
Polyacrylic acid, linear; aqueous solution 35% (w/w)	PAA	Sigma Aldrich, USA	#0000181748
Polystyrene sulfonic acid; aqueous solution 30% (w/V)	PSSA	Thermo Scientific, USA	#W10G005
Shellac SSB57 Luna, FL	Shellac	SSB Stroeve, Germany	#271400
Polyvinyl acetate phthalate Opadry Enteric 91 series	PVAP	Colorcon, USA	#TKL60934
Methacrylic acid – ethyl acrylate copolymer (1:1) Type B (partially neutralised) Kollicoat MAE 100P	MAE-B	BASF, Germany	#02092416K0
Polyethylene glycol 6,000 Macrogol	PEG	AppliChem, Germany	#OCOO3513
Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer Soluplus	SOLU	BASF, Germany	#77868636WO
Methyl methacrylate and diethylaminoethyl methacrylate copolymer Kollicoat Smartseal 100P	Smartseal	BASF, Germany	#B120603009

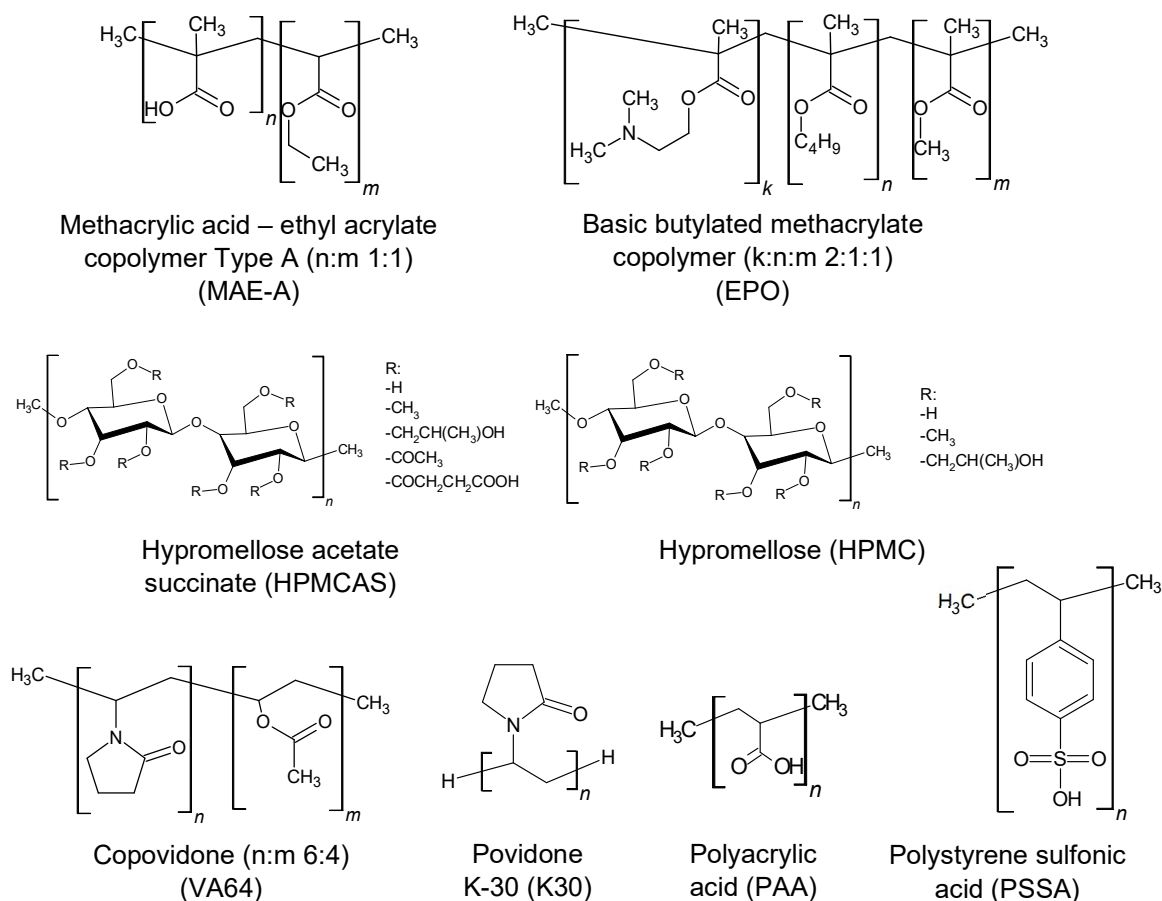


Figure 3.2 Chemical structure of the main polymers used in this work. The respective abbreviations are given in brackets.

As PAA and PSSA were purchased as aqueous solutions, they were not used in ASD-preparation techniques, but only in solubility enhancement testing and in molecular modelling. The preparation of a powdered form via aqueous spray drying worked for both polymers, but as they were not soluble in organic solvents, no spray drying with the APIs was performed. Due to their high acidity, they were not used in extrusion to not damage the screws or heating block material.

3.1.3 Miscellaneous substances and reagents

The further substances and reagents used in this work are listed in Table 3.2. With these chemicals, various buffer solutions were prepared:

Buffer preparation for solubility and partition coefficient testing (phosphate buffer pH 6.8 R, 0.15 M)

Phosphate buffer pH 6.8 R was used (Ph. Eur. 10.0). The buffer consisted of 0.15 M Na₂HPO₄ and 0.02 M citric acid, prepared by dissolving Na₂HPO₄ · 2H₂O and C₆H₈O₇ · H₂O in HPLC grade water. An adjustment of the pH was not necessary.

Fasted state simulated intestinal fluid (FaSSIF pH6.8)

Fasted state simulated intestinal fluid (FaSSIF) was prepared from sodium taurocholate (3 mM), lecithin from soybean (0.75 mM), sodium dihydrogen phosphate monohydrate (30 mM), and sodium chloride (105 mM). To adjust the pH to 6.8, sodium hydroxide was added in the required amount. The medium was used for solubility testing and dissolution experiments in the USP II apparatus. For medium exchange experiments, a concentrate was prepared with eleven-fold increased molarities of the components. Sodium hydroxide was added in a concentration of 0.1 M to the concentrate to neutralise the hydrochloric acid in the medium. After dissolution in 200 mL 0.01 M HCl, 20 mL of this concentrate were added to achieve the above-mentioned concentrations in a total volume of 220 mL.

Buffer preparation for TFAM analysis and polymer dissolution rate (FaSSIF pH 6.8 buffer)

The dissolution medium in the tangential flow absorption model and in the turbidity analysis of polymer dissolution rate was 30 mM phosphate buffer with 105 mM sodium chloride pH 6.8, equivalent to FaSSIF pH 6.8 without the addition of lecithin and sodium taurocholate. The pH was adjusted with sodium hydroxide in the required amount.

Table 3.2 Further substances and reagents used in this work.

Substance / trade name	Abbreviation / synonym	Supplier	Batch / lot
Acetonitrile, HPLC grade	ACN	Fisher Chemical, USA	various
Bovine serum albumin, Fraction V pH 7.0	BSA	AppliChem, Germany	#OVO13516
Citric acid monohydrate	C ₆ H ₈ O ₇ · H ₂ O	Carl Roth, Germany	#066238855
Chloroform, deuterated	Chloroform-d, CDCl ₃	Deutero, Germany	#B24854
Dimethyl sulfoxide, > 99.5%	DMSO	Carl Roth, Germany	#445863447
Dimethyl sulfoxide, deuterated 99.8% + 0.03% TMS	DMSO-d ₆	Deutero, Germany	#25441 #00905
Disodium hydrogen phosphate dihydrate	Na ₂ HPO ₄ · 2H ₂ O	Carl Roth, Germany	#448272158
Ethanol, HPLC grade	EtOH	Fisher Chemical, USA	various
Hydrochloric acid, 37%	HCl, 37%	Fisher Chemical, USA	#1875199
Hydrochloric acid, deuterated, 35%	DCl, 35%	Sigma Aldrich, USA	#MKCG2538
Lecithin from soybean	-/-	Thermo Scientific, USA	#N02J039
Lidocaine, Ph. Eur. 9.0	Lido	Caelo, Germany	#19054505
Methanol, deuterated + 0.03% TMS	MeOD, MeOH-d ₄	Deutero, Germany	#25880
Methanol, HPLC grade	MeOH	Fisher Chemical, USA Honeywell, USA	various
Octanol	-/-	Honeywell, USA	#K3510 #K0150
ortho-phosphoric acid, 85% (w/w)	-/-	Sigma Aldrich, USA Honeywell Fluka, USA	#MKCB7708 #SZBG2860H

Substance / trade name	Abbreviation / synonym	Supplier	Batch / lot
Sodium chloride, 99.5%	NaCl	Fisher Chemical, USA	#1686186
Sodium dihydrogen phosphate monohydrate	NaH ₂ PO ₄ · H ₂ O	AlfaAesar, USA	#R22A024
Sodium hydroxide	NaOH	VWR Chemicals, USA	#185254102 #18H254102 #14H370042
Sodium taurocholate	-/-	Thermo Scientific, USA	#Z19J040 #P27J018
Tert-butyl alcohol, 99.5%	-/-	Acros Organics, USA	#A0444137
Triethylamine, 99%	TEA	Thermo Fisher, USA	#10235518
Toluene, deuterated	Toluene-d ₈	Deutero, Germany	#23481
Water, deuterated	D ₂ O	Deutero, Germany	#B25024
Water, HPLC grade	H ₂ O	Fisher Chemical, USA, or freshly prepared	various

3.2 Methods

3.2.1 ASD preparation methods

3.2.1.1 *Preparation of physical mixtures*

Physical mixtures of the active substances and the polymers were prepared by manually blending different ratios of API and polymer using a mortar and pestle for at least five minutes. For subsequent DSC analysis and preparation of ASDs inside the DSC via melt quenching, mixtures with an active ingredient content of 10%, 30%, 50%, 70%, 80% and 90% (w/w) were prepared. The mixtures with cinnarizine contents of 10% and 50% (w/w) were used in the preparation of discs by vacuum compression molding (VCM; see section 3.2.1.5). Additionally, selected physical mixtures of both APIs were processed through HME.

3.2.1.2 *Hot melt extrusion*

Hot melt extrusion was done using a 9 mm co-rotating hot melt extruder (20:1) with three heating zones and a 2 mm single circular orifice die (ZE 9 HMI, ThreeTec, Switzerland). The temperature and speed settings were varied depending on the thermal properties of the processed API and polymer (see Table 3.3). A total of 10 g powder mixture was used for each run. Feeding was done manually as well as pulling the extrudates from the nozzle to obtain filaments.

Milling of the extrudates was performed depending on the requirements of the analytical method. For the determination of the API content, DSC and XRPD, extrudates were milled manually using a mortar and pestle. For dissolution testing and particle size determination a planetary ball mill (Model PM100, Retsch, Germany) was utilised. Ball milling was performed with a 25 mL sample cup and eight milling spheres. Milling time and speed were varied in a range of 3 to 15 minutes and 300 to 600 rpm, respectively, depending on the material hardness of the extrudates. Extrudates with an elasticity too high to be grinded by the ball mill were milled using a cryomill (CryoMill, Retsch,

Germany). A 20 mL sample cup with one milling sphere was constantly cooled using liquid nitrogen prior to and during the milling process.

Table 3.3 Settings for hot melt extrusion for cinnarizine- and trimethoprim-polymer systems.

Active substance	Polymer	Drug loading [%]	Temperature HME [°C]			Screw speed [rpm]
			1	2	3	
Cinnarizine	-/-	100	105	120	120	40
	MAE-A	10	110	160	160	45
		30	110	160	160	45
		50	110	160	160	45
		50	110	160	160	45
	EPO	10	110	130	130	25
		30	110	130	130	25
		50	110	130	130	25
	HPMCAS	10	110	160	160	40
		30	110	160	160	40
		50	110	160	160	40
	HPMC	10	110	160	160	25
		30	110	160	160	25
		50	110	160	160	25
	VA64	10	100	160	160	25
		30	100	160	160	25
		50	100	130	130	25
	K30	10	110	160	160	15
		30	110	160	160	20
		50	110	160	160	30
Trimethoprim	MAE-A / VA 1:1		50	110	160	20
	HPMCAS / VA 1:1		50	110	160	25
	-/-	100	110	210	210	25
	MAE-A	10	110	160	160	15
		30	110	160	160	15
		50	110	160	160	15
		50	110	160	160	15
	EPO	10	110	210	210	25
		30	110	210	210	25
		50	110	210	210	25
	HPMCAS	10	110	210	210	25
		30	110	210	210	25
		50	110	210	210	25
	HPMC	10	110	210	210	25
		30	110	210	210	25
		50	110	210	210	25
		70	110	210	210	25
	VA64	10	110	210	210	20
		30	110	210	210	20
		50	110	210	210	20
		70	110	210	210	20
	K30	10	110	165	165	15
		30	110	165	165	15
		50	110	165	165	15
	HPMCAS / VA 1:1	30	110	210	210	20
		50	110	210	210	20
		70	110	210	210	20

For extrudates prepared with cinnarizine, the melting temperature of 120 °C was exceeded in all cases, leading to a completely molten system in the extruder. Due to the higher melting temperature of 202 °C for trimethoprim, the temperature of zone 2 and 3 in the extruder was kept below the melting point when using MAE-A and K30 as polymers due to their low degradation temperature. Here, 160 °C and 165 °C were chosen, respectively. Although melting of the API was not possible with MAE-A and K30, an attempt was made to embed the active ingredient in the polymers by means of increased mechanical forces. For this purpose, screws with additional mixing and kneading elements were used. The configuration is shown in Figure 3.3.



Figure 3.3 Screws used in hot melt extrusion. Top: Conveyor screw. Bottom: Screw with additional kneading and melting elements.

3.2.1.3 Spray drying

Spray drying was performed on an S-300 Mini Spray Dryer with S-395 inert loop in closed mode (both Büchi, Switzerland). A high efficiency cyclone was used as only small batch sizes were manufactured. The setup was equilibrated for one hour before starting to spray the sample solution. After the run, the powder was stored in a vacuum drying cabinet (VDL 53, Binder, Germany with MZ 2C EX+AK+EK, Vacuubrand, Germany) at 40 °C and < 20 mbar for one hour for subsequent solvent removal before performing the DSC and XRPD analyses and content determination.

For cinnarizine, a total batch size of 4 g solid substance was targeted. Due to the solubility of 8 mg/mL for cinnarizine in methanol, the solvent volume was adjusted accordingly, leading to the following compositions:

- 30% drug load (w/w): 1.2 g cinnarizine + 2.8 g polymer dissolved in 150 mL of methanol
- 50% drug load (w/w): 2.0 g cinnarizine + 2.0 g polymer dissolved in 250 mL of methanol
- 70% drug load (w/w): 2.8 g cinnarizine + 1.2 g polymer dissolved in 350 mL of methanol

For all drug load and polymer combinations, the settings were 90 °C inlet temperature, 5 mL/min pump speed, 23 m³/h drying gas, and 600 L/h spray gas, resulting in outlet temperatures of 70 – 80 °C. Systems with MAE-A (50% and 70% drug load (w/w)), HPMCAS (30% and 50% drug load (w/w)), HPMC (30% and 50% drug load (w/w)), VA64 (10%, 30%, 50% and 70% drug

load (w/w)), K30 (30% and 50% drug load (w/w)), Soluplus (30% drug load (w/w)) and a 1:1 mixture of MAE-A and VA64 (50% drug load (w/w)) were prepared. EPO was not used due to its low glass transition temperature resulting in particles sticking to the glass walls of the cyclone.

For trimethoprim, 1.4 g of solid substance were dissolved in 200 mL methanol prior to spray drying, corresponding to a solid concentration of 0.7% (m/V) for each combination. The settings of the spray dryer were adjusted to increase product yield. Table 3.4 summarises the settings for the single runs with trimethoprim.

Table 3.4 Settings for the spray drying experiments with trimethoprim and various polymers.

Polymer	Drug loading [% (w/w)]	Drying gas [m ³ /h]	Spray gas [L/h]	Pump rate [mL/min]	Inlet temperature [°C]
-	100	23	800	5.0	70
MAE-A	30	22	800	5.0	70
	50	24	800	5.0	70
	70	24	800	5.0	70
EPO	30	22	800	5.0	70
	50	21	850	5.0	70
HPMCAS	30	22	800	5.0	70
	50	22	800	3.0	80
	50	24	800	5.0	90
	70	22	800	3.0	70
HPMC	30	20	700	5.0	80
	50	22	750	5.0	80
	50	24	800	5.0	90
	70	22	800	3.0	70
VA64	30	23	800	5.0	70
	50	23	800	5.0	70
	70	23	800	5.0	70
K30	30	24	800	5.0	70
	50	24	800	5.0	70
	70	24	800	5.0	70

3.2.1.4 Melt quenching

Melt quenching was done together with DSC analysis of the physical mixtures. In a first heating cycle, water was evaporated through non-hermetic lids. The samples were heated above the melting temperature of the API (except for trimethoprim-MAE-A and trimethoprim-K30 combinations due to polymer degradation below the API's melting point). The ASDs were prepared by subsequent rapid cooling with 20 K/min down to -20 °C. The upper limits are listed in Table 3.5. During the second heating cycle, the thermal characteristics of the melt-quenched systems were analysed, as recrystallisation or melting events. Further information on the DSC settings can be found in chapter 3.2.3.2.

Table 3.5 Upper limits for the melt quenching of cinnarizine-polymer and trimethoprim-polymer physical mixtures to obtain ASDs.

API	MAE-A	EPO	HPMCAS	HPMC	VA64	K30
Cinnarizine	160 °C	150 °C	200 °C	200 °C	200 °C	165 °C
Trimethoprim	160 °C	220 °C	220 °C	220 °C	220 °C	165 °C

3.2.1.5 Vacuum compression molding

For vacuum compression molding, a MeltPrep VCM Tool (Melt Prep, Austria) with a D20 piston and insert was used. 200 mg of the powder blend were added to the sample chamber covered with a polytetrafluoroethylene foil before a vacuum was applied. Temperature and duration of the melting process were varied depending on the polymer (Table 3.6). After melting, the sample chamber was cooled using compressed air and water-cooling.

Table 3.6 Settings for the vacuum compression molding of cinnarizine-polymer combinations.

	Drug load [% (w/w)]	MAE-A	EPO	HPMC AS	HPMC	VA64	K30
Temperature [°C]	10	160	130	160	160	160	165
	50	160	130	160	160	130	160
Duration [min]	10	10	10	10	15	10	10
	50	10	10	10	10	10	10

3.2.1.6 Milling

Ball milling was done with a PM 100 planetary ball mill (Retsch, Germany) and sample cups of 25 mL with eight milling spheres of 10 mm diameter. The cups were filled with 8 mL of the respective substance or physical mixture of API and polymer. The milling time was either 180 or 360 minutes at 600 rpm with a change of direction in 20-minute-intervals. For 180 minutes, the following samples were milled: crystalline trimethoprim, trimethoprim in VA64 (10%, 20%, and 30% drug load (w/w)), trimethoprim in HPMCAS (5% and 10% drug load (w/w)) and trimethoprim in MAE-A (15% and 20% drug load (w/w)). 5% trimethoprim in HPMCAS and 20% in MAE-A were also milled for 360 minutes. Cinnarizine physical mixtures were not milled in the ball mill to obtain ASDs due to cinnarizine's low glass transition temperature. Samples from hot melt extrusion were grinded by ball milling with the same apparatus for 3 to 15 minutes at 300 to 600 rpm depending on the material hardness to obtain a powder for subsequent analysis.

3.2.1.7 Film casting and high-throughput screening

To screen the polymers in regards to their suitability to form amorphous solid dispersions with trimethoprim at varying drug loads, film casting and high-throughput screening with subsequent analysis via a polarised light microscope were applied. For film casting, solutions of trimethoprim and the respective polymer in methanol with a total solids content of 0.7% (w/V) were coated onto glass plates with a four-sided film casting knife (KR 3000, mtv Messtechnik, Germany) and a layer thickness of 100 µm. The glass plates were dried in a vacuum drying cabinet at 40 °C and < 20 mbar

(VDL 53, Binder, Germany with MZ 2C EX+AK+EK, Vacuubrand, Germany) or in a laminar airflow box (LAF) at room temperature. The drug load in the solid was 30%, 50%, and 70% (w/w), respectively.

For high-throughput screening, stock solutions of trimethoprim and the polymer (each 0.35% (w/V)) were pipetted into transparent 48-well plates in changing ratios to obtain a total volume of 200 μ L in each well. The drug loads ranged from zero to 100% (w/w) in increments of ten. Pipetting and drying were done in the laminar airflow box. The assessment of both the glass plates and the well plates was performed visually by polarised light microscopy (OPO 185, Kern & Sohn, Germany). The samples with the highest amorphous drug load found in the high-throughput screening were prepared on a larger scale by solvent evaporation. The solutions with a total solids content of 1.2% (w/V) were prepared in methanol (100 mL) and dried in the vacuum drying cabinet at 40 °C and < 20 mbar over night. For this purpose, the solutions were poured into silicone wells with the dimensions of 4 · 7 · 2 cm. Each solution was filled into four wells with a fill height of about 0.9 cm. After drying, the films were removed from the wells, milled in the ball mill and analysed using XRPD.

3.2.2 Salt preparation

To analyse the protonation behaviour of cinnarizine, the hydrochloride salt was prepared by spray drying and vacuum drying. As two possible protonation sites are available in the molecule, a 1:1 ratio and a 1:2 ratio of cinnarizine and hydrochloric acid regarding the number of molecules were prepared.

In spray drying, the same setup as described in section 3.2.1.3 was used with 24 m³/h drying gas, 95 °C inlet temperature, 500 L/h spray gas, and 5.0 mL/min pump speed, resulting in an outlet temperature of approximately 63 °C. Afterwards, the products were dried in a desiccator for 1.5 hours prior to analyses. For the 1:1 ratio (1:2 ratio), 10 g of cinnarizine (5 g of cinnarizine) were dissolved in 1.25 L of methanol and 2.265 mL of 37% hydrochloric acid were added (equal to 2.673 g due to a density of 1.18 g/mL). The pH of the solution was 5 for the 1:1 ratio and 2-3 for the 1:2 ratio, determined with indicator paper.

For vacuum drying, 800 mg of cinnarizine were dissolved in 100 mL of methanol before adding either 178 μ L (1:1 ratio) or 364 μ L (1:2 ratio) of 37% hydrochloric acid. The solutions were sonicated for 15 minutes to dissolve the API and then poured into silicone wells with the dimensions of 4 · 7 · 2 cm (divided in 4 · 25 mL per well). Drying was performed in a drying cabinet at 40 °C and < 20 mbar (VDL 53, Binder, Germany with MZ 2C EX+AK+EK, Vacuubrand, Germany) for three hours.

The spray-dried samples were tested with the equilibrium shake flask method for thermodynamic solubility in phosphate buffer pH 6.8 *R*, water and octanol, and XRPD spectra were recorded. Both

the spray-dried and vacuum-dried samples were analysed in non-hermetic pans in the DSC. ¹H-NMR spectra were recorded for all samples in methanol-d₄, DMSO-d₆, and chloroform-d.

3.2.3 Solid powder analytics

3.2.3.1 Thermogravimetric analysis

Thermogravimetric analysis (TGA) using a TGA 2 (Mettler Toledo, Germany) was performed for the raw materials to determine the onset of the degradation temperature by weight-loss. To create an inert atmosphere, the sample chamber was purged with nitrogen at a flow rate of 50 mL/min. A sample of about 10 mg was weighted in aluminium oxide crucibles and exposed to a heating ramp of 20 K/min in the range between 40 °C and 600 °C. Evaluation was done using the STARExcellence-Software (version 16.40, Mettler Toledo, Germany). The analysis was performed with $n = 3$ samples to calculate the mean, the standard deviation and the relative standard deviation. As the detected degradation temperature is referring to the presence of volatile substances, a potential earlier onset of degradation without weight-loss cannot be excluded. Therefore, DSC analysis, melt quenching, and hot melt extrusion were not performed at the detected degradation temperature, but at lower temperatures to maintain a safety margin. This accounted also for differences in nitrogen atmosphere measurements in TGA and DSC compared to oxygen containing air in extrusion processes.

3.2.3.2 Differential Scanning Calorimetry

DSC analysis of the raw materials, physical mixtures and ASDs was performed on a DSC Q2000 (TA Instruments, USA) in combination with the evaluation software TA Universal Analysis 2000 (version 4.5A). Pans and lids, both hermetic and non-hermetic were of the Tzero-series (Waters, USA). As a reference, an empty pan with lid was used for each measurement and the sample mass varied between 5 and 20 mg. All samples underwent a heat-cool-heat cycle with a heating ramp of 10 K/min and a cooling ramp of 20 K/min with -20 °C as the lower limit. The upper limit was chosen to be below the polymer degradation temperature (see Table 3.5). After each ramp, the sample was equilibrated at the given temperature for three minutes. The purge gas was nitrogen with a flow rate of 50 mL/min. The glass transition temperature was determined as the inflection point of the glass transition, while the maximum and minimum of the curve were used for crystallisation and melting temperatures, respectively. While onset values are also commonly reported, T_{max} was selected for consistency with previous work and because it provides a reproducible descriptor of the melting transition. Furthermore, this procedure is well-described in literature [139–141].

For the raw materials and physical mixtures, non-hermetic lids were used for any residual water to evaporate during the first heating cycle. Consequently, the second heating cycle provided the glass transition temperatures of the melt quenched samples without the plasticizing effects of water.

The crystalline fraction was quantified from the DSC plots based on the area under the melting peak, which is equal to the melting enthalpy. By measuring the enthalpy for known crystalline

contents (initial heating of physical mixtures of crystalline drug and polymer), a calibration line was obtained to derive the percentage of crystallinity for the individual samples. If recrystallisation occurred during the heating in the DSC prior to melting, the recrystallisation enthalpy was subtracted from the melting enthalpy before calculating the degree of crystallinity.

All DSC measurements to assess the glass transition temperature, the melting temperature, and the melting enthalpy as well as potential crystallisation signals were performed in triplicates and the mean, the standard deviation, and the relative standard deviation were calculated.

3.2.3.3 XRPD analysis

X-ray powder diffraction was analysed with a D2 phaser with a copper anode (Bruker, USA). The measurement range was 5 ° to 50 ° two theta with a width of 0.024 two theta and a time of 0.3 seconds per step. Powders and milled extrudates were placed on 51.5 mm sample holders, which rotated with ten rounds per minute during the measurement. Evaluation was performed using the software DIFFRAC.EVA (version 6.1.0.4, Bruker, USA).

3.2.3.4 Helium pycnometry

The density of the raw materials was determined using an AccuPyc II 1340 helium pycnometer (Micromeritics, Germany). A sample cup of 3.5 cm³ with a porous lid was used to analyse powders of 1.0 – 1.5 g. The helium pressure setting was 19.500 psig and ten purge cycles were applied prior to each measurement with ten additional cycles and an equilibration time of ten seconds. Before conducting the measurements, a calibration with a standardised metal ball was performed. The measurement was performed in triplicates to calculate the mean, the standard deviation, and the relative standard deviation.

3.2.3.5 Dynamic vapour sorption

Dynamic vapour sorption (DVS) was used to perform a stress test of chosen prepared ASDs. Samples were transferred into aluminium wells of the SPSx-1 μ High Load DVS (ProUmid, Germany). A conditioning phase at 40 °C and 0% relative humidity was used to eliminate any humidity from the samples and to determine the weight. Then, the relative humidity was increased to 75% with a ramp of 6.25% per hour. The change in the sample mass was determined over time until an equilibrium of 0.01% mass change per 50 minutes was reached. Then, the humidity was decreased again to 0% to dry the samples.

3.2.3.6 Particle size determination

A Kern OPO 185 microscope was used (Kern & Sohn, Germany) to take pictures of the milled extrudates, raw materials, or spray-dried products. Particles were counted (at least 500 particles per sample) and measured using the software ImageJ (version 1.54J, NIH, USA) with an automated macro to determine Feret's diameter. With the results, q₀-plots were created to compare the particle size distribution of the different systems.

3.2.4 Solution characterisation

3.2.4.1 Content determination via liquid chromatography

40 mg of the hand-milled extrudates or spray-dried powders were dissolved in 20 mL of methanol and stirred over night or put into an ultrasonic bath until completely dissolved. Prior to HPLC (high-performance liquid chromatography) analysis, the solutions were diluted with methanol to obtain a final target concentration between 0.1 and 0.5 mg/mL for cinnarizine or between 0.1 and 0.2 mg/mL for trimethoprim. All measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

HPLC was performed on a DGU-20A3 degasser, an LC-20AD pump, a SIL-30ACMP autosampler, a CTO-20AC column oven, an SPD-M20A diode array detector and a CBM-20A communications bus module (Shimadzu, Japan) with a 4.6 x 250 mm XTerra RP18 5 μ m column (Waters, USA). The oven temperature was 40 °C, the flow rate was 0.5 mL/min. For the analysis of cinnarizine, the mobile phase consisted of methanol, acetonitrile and 0.1% triethylamine (8:1:1, pH 7.8 adjusted with ortho-phosphoric acid) and the run time was 12 minutes. Quantitation was done by UV absorption at 249 nm and external calibration (0.01 - 1000 μ g/mL). For Trimethoprim, the mobile phase consisted of methanol and purified water (6:4, pH 3.0 adjusted with ortho-phosphoric acid) as mobile phase. The run time was 8 minutes, and quantitation was performed via UV absorption at 204 nm using external calibration over a concentration range of 0.1 – 250 μ g/mL. Exemplary chromatograms are shown in the appendix (Figure 8.1 and Figure 8.2).

3.2.4.2 Solubility testing

The saturation solubility of the active ingredients was determined by adding an excess of the active to different aqueous and organic media, including hydrochloric acid at various concentrations, phosphate buffer pH 6.8 R, FaSSIF pH 6.8, methanol, ethanol, octanol, DMSO, acetonitrile and tert-butyl alcohol. The influence of the polymers on the saturation solubility was tested by dissolving 10 mg/mL (1%) or 50 mg/mL (5%) of polymer in phosphate buffer prior to the addition of the active ingredient. The mixtures were stirred for at least 72 hours. The pH was measured at the beginning and the end of the stirring period. Afterwards, the samples were centrifuged (12,000 rpm for 3 minutes) and diluted (1 mL + 1 mL of the respective solvent). The concentration of the dissolved drug was determined by HPLC analysis (see 3.2.4.1). All measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

3.2.4.3 Partition coefficient

The partition coefficient was determined similar to a standard $\log P$ measurement, but with varying composition of the aqueous phase including different polymer solutions, acids and buffers. The drug was dissolved in octanol prior to the experiment. Then, 10 mL of the aqueous media were filled into tubes and layered with 10 mL of the octanol phase with dissolved API. To avoid the distribution and precipitation of polymers or buffer salts in the octanol phase, the tubes were neither

shaken nor stirred. After 72 hours, aliquots from both phases were taken and treated as described above in the solubility testing. The pH of the aqueous phase was measured at the beginning and at the end of the procedure. All measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

3.2.4.4 Dissolution testing – USP II

Dissolution testing was performed in a USP II paddle apparatus (DT 128 light by Erweka, Germany) with FaSSIF pH 6.8 at 37.0 °C stirred at 50 rpm for four hours. Samples were taken after 1, 5, 10, 15, 30, 60, 90, 120, 180 and 240 minutes and centrifuged at 12,000 rpm for 3 minutes. Prior to HPLC analysis according to the method described in 3.2.4.1, the supernatant was diluted 1:1 (V/V) with methanol. To maintain a constant dissolution volume, each removed 1 mL aliquot was replaced with fresh FaSSIF medium. The analysed samples are listed in Table 3.7. VCM discs with a total weight of 200 mg with 10% (w/w) and 50% (w/w) drug load of cinnarizine were analysed as well as 200 mg of the 10% and 50% loaded milled extrudates. For trimethoprim, only milled extrudates were analysed with drug loads of 10% (w/w) and 50% (w/w). For 10% drug load, both 200 mg and 2000 mg were analysed. For 50% drug load, 2000 mg were used. As HPMC extrudates were too flexible for milling at all drug loads in both the ball mill and the cryomill, they were not analysed.

Table 3.7 Conducted dissolution experiments with different setups for cinnarizine and trimethoprim ASDs from hot melt extrusion (HME), spray drying (SD) and vacuum compression molding (VCM).

Active substance	Polymer	Samples	USP II	USP II with pH shift	TFAM
Cinnarizine	-/-	Neat API, 20 mg and 100 mg	X		
		Extruded API, 20 mg	X		
	HPMCAS	VCM 10% and 50%	X		
		HME 10%	X	X	
		HME 50%	X		
	HPMC	VCM 10% and 50%	X		
		HME 10% and 50%	X		
	EPO	VCM 10% and 50%	X		
		HME 10%	X	X	
		HME 50%	X		
	MAE-A	VCM 10% and 50%	X		
		HME 10%	X	X	
		HME 50%	X		
		SD 50%	X		
	VA64	VCM 10% and 50%	X		
		HME 10%	X	X	
		HME 50%	X		
		SD 50%	X		
	K30	VCM 10% and 50%	X		
		HME 10% and 50%	X		
	MAE-A / VA64 1:1	HME 50%	X		
		SD 50%	X		
	HPMCAS / VA64 1:1	HME 50%	X		

Active substance	Polymer	Samples	USP II	USP II with pH shift	TFAM
Trimethoprim	-/-	Neat API, various amounts	X		X
		HME 10%, 200 mg and 2000 mg	X		
	HPMCAS	HME 50%, 2000 mg	X		X
		SD 30% and 50%			X
		SD 30%			X
	HPMC	SD 30%			X
		SD 30%			X
	EPO	HME 10%, 200 mg and 2000 mg	X		
		HME 50%, 2000 mg	X		
	MAE-A	HME 10%, 200 mg and 2000 mg	X		
		HME 50%, 2000 mg	X		
		SD 30% and 70%			X
	VA64	HME 10%, 200 mg and 2000 mg	X		
		HME 50%, 2000 mg	X		X
		SD 30%			X
	K30	HME 10%, 200 mg and 2000 mg	X		
		HME 50%, 2000 mg	X		
		SD 30%			X
	HPMCAS / VA64 1:1	HME 50%, 2000 mg	X		

In pH shift experiments, the first dissolution step was performed in 200 mL of 0.01 M hydrochloric acid with sampling at 1, 5, 10, 15, 30, 60, 90, and 120 minutes. The FaSSIF pH 6.8 concentrate with 0.01 M hydroxide addition was then added with a volume of 20 mL. Sampling was then continued at 1, 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes after addition, leading to a total run time of 360 minutes. The removed aliquots were substituted by either 0.01 M HCl or FaSSIF pH 6.8. The centrifugation and dilution with methanol was equal to the setup without pH shift.

If not stated otherwise, all measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

3.2.4.5 Dissolution testing – TFAM

The dissolution medium in the tangential flow absorption model was 30 mM phosphate buffer pH 6.8, equivalent to FaSSIF pH 6.8 without the addition of lecithin and sodium taurocholate (see chapter 3.1.3). The tangential flow filtration unit was a Vivaflow 50 R Hydrosart membrane with a molecular weight cut-off (MWCO) of 5 kDa and a surface area of 50 cm². The whole setup is displayed in Figure 3.4. Prior to the experiment, the system was purged with water and then phosphate buffer. The inlet and outlet flow was calibrated to 1.0 mL/min. At t = 0 minutes, the powdered sample with an API content of about 430 mg was added into the dissolution compartment together with 50 mL of dissolution medium to start the experiment. By this, a 100% drug release would be equal to a tenfold supersaturation. During the total experimental duration time of 240 minutes, the fill level in the dissolution compartment was monitored and the outlet medium was collected in a graduated cylinder in order to adjust the pump if required. At 1, 5, 10, 15, 30, 60, 90, 120, 180 and 240 minutes, samples of 3 mL were collected in tubes containing 9 mL of methanol for dilution and preventing precipitation from a supersaturated solution. The diluted samples were analysed via an EVOLUTION 220 UV-Vis spectrometer (Thermo Scientific, USA) with the software

ThermoInsight (Version 2.5.92, Thermo Scientific, USA) at 276 nm in a concentration range of 7 - 47 $\mu\text{g/mL}$ (additional dilution done if needed). With the TFAM, samples from spray drying were analysed as well as selected formulations from hot melt extrusion. If not stated otherwise, all measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

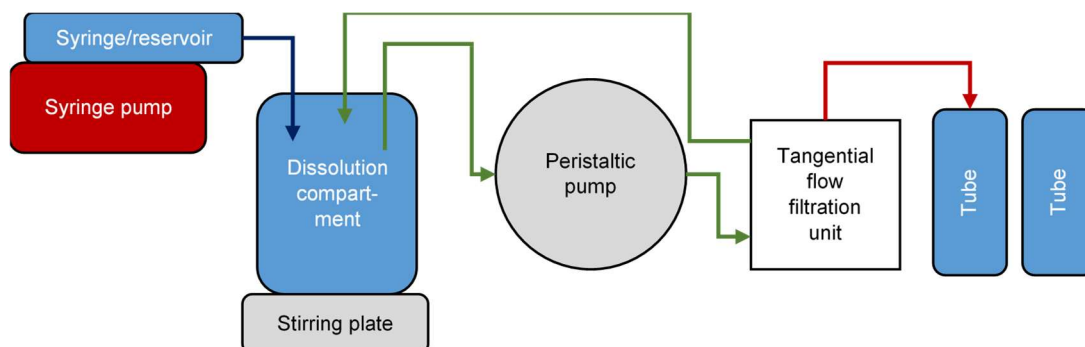


Figure 3.4 Schematic diagram of the tangential flow absorption model. The medium from the reservoir is added to the dissolution vessel by a syringe pump, with the volume loss due to filtration being matched. The permeate is collected in tubes containing a fixed amount of methanol to hinder the precipitation of trimethoprim. Figure adapted from Haering et al. [99] with permission.

3.2.4.6 Dissolution testing – polymer dissolution speed

The speed of polymer dissolution was assessed for the polymers MAE-A, EPO, HPMCAS, HPMC, VA64 and K30 in FaSSIF pH 6.8 buffer via turbidity analysis. A fixed amount of polymer in powdered form was given into the buffer, and the turbidity was monitored in defined intervals for 240 minutes. The samples were stirred continually at room temperature within the cuvettes. Recording of the absorbance was done at 660 nm using an EVOLUTION 220 spectrometer (Thermo Scientific, USA) with the software ThermoInsight (Version 2.5.92, Thermo Scientific, USA) with FaSSIF pH 6.8 buffer as a blank. If not stated otherwise, all measurements were performed in triplicates and the mean, standard deviation, and relative standard deviation were calculated.

3.2.4.7 $^1\text{H-NMR}$

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 400 MHz magnet (Bruker, USA). The samples in deuterated solvents were analysed in 5 mm tubes (Boro400-5-7, deuterio, Germany) with 16 scans each at 298 K. Raw materials were analysed with ^1H -spectra as well as mixtures of active ingredient and polymers in varying ratios. Evaluation was done using the software TopSpin (version 4.1.4, Bruker, USA).

To compare different solvents, cinnarizine physical mixtures with the selected polymers were dissolved in either methanol- d_4 or toluene- d_8 . The drug content relative to the polymer was adjusted to 10%, 50%, and 90% (w/w). Titration experiments were performed with a constant polymer concentration and increasing active ingredient concentration by adding a mixture of both to a

polymer solution in small steps (10 – 250 μ L), each time recording a ^1H -NMR spectrum. Concentrations were based on solubility and detection limits.

Cinnarizine titrations were performed in methanol- d_4 . The API concentrations ranged from 0.4 mM to 11.3 mM and the API-polymer ratios were 6 to 300. The polymer concentrations were about 60 μ M for HPMCAS, HPMC, EPO, VA64, and K30. The concentration for MAE-A was 30 μ M due to its high molecular weight. For titration experiments with deuterated hydrochloric acid (DCl) instead of a polymer, the cinnarizine concentrations were 0.1 mM to 5.8 mM and DCl solutions were used in concentrations of 0.06 mM, 0.6 mM, and 6.0 mM, covering ratios of 0.02 to 96.

Trimethoprim titrations were done with both deuterated methanol and deuterated DMSO as solvent. The API concentrations were in a comparable range of 0.4 mM to 5.7 mM and polymer concentrations were 60 μ M (except for MAE-A with 40 μ M), leading to API-polymer ratios of 7 to 150. For a better comparison of the results with literature findings from other methods, the lidocaine titration was performed in D_2O instead of an organic solvent; also, the solubility of BSA in required amounts was not given in deuterated methanol. Lidocaine was titrated from 0.2 μ M to 4.5 μ M with a constant BSA concentration of 80 μ M to cover ratios from 2 to 60.

The titration experiments were performed with $n = 3$ for each API-polymer combination to calculate the mean, standard deviation and relative standard deviation.

3.2.5 Stability testing

To assess the long-term stability of the extruded amorphous solid dispersions with cinnarizine at different storage conditions, the hand-milled extrudates were placed into three transparent glass vials sealed with rubber stoppers and parafilm. As storage conditions, refrigeration at 8 $^{\circ}\text{C}$, 25 $^{\circ}\text{C}$ / 60% relative humidity and at 40 $^{\circ}\text{C}$ / 75% relative humidity were chosen. Sampling time points were 3, 6, 9, 12, 18 and 24 months. The stored samples were analysed by DSC and XRPD for the presence of crystallinity. If crystallinity was present in the samples, the crystalline portion was quantified by the melting enthalpy as described in 3.2.3.2.

3.2.6 Calculations

3.2.6.1 Phase diagrams

To construct phase diagrams of cinnarizine and trimethoprim with different polymers, glass transition temperatures of ASDs prepared by melt quenching in the DSC were measured. Physical mixtures of the drug and the polymer were heated to melt the active ingredient and to soften the polymer. After an equilibration time of three minutes, the sample was cooled rapidly to create the ASD. In the second heating cycle, the T_g of the mixed system was obtained as well as signals showing any residual crystallinity or recrystallisation. The solubility of the active ingredient in the polymer was assessed via the melting point depression, being the shift of the melting point of the active with increasing polymer concentration [32,40,133] as shown in Figure 3.5. Heating was done with

2 K/min, 5 K/min, and 10 K/min to extrapolate a melting point onset for a theoretical heating rate of 0 K/min, which was used as the solubility temperature for further calculations. In scientific literature, there are studies using either the onset [81,142–144] or the offset [63,65,70] of the melting peak as solubility temperature with no final decision which method is more adequate. In this study, it was decided to use the onset of the melting peak, which is the more common approach as stated in a paper by Medarević et al. [144].

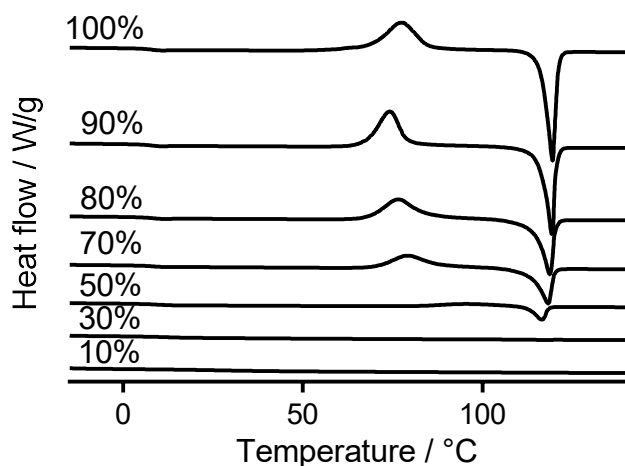


Figure 3.5 Thermograms of cinnarizine-EPO systems prepared by melt quenching with indicated drug load. Datasets are stacked on the y-axis (heat flow) for better visualisation. Exothermal events are plotted upwards. The data shown is obtained from the first heating cycle after melt quenching, showing the melting point depression.

In a first attempt with cinnarizine, it was assessed which of the established and less known methods to calculate glass transition and solubility curves fit best to the experimental results. The theoretical glass transition temperatures were calculated based on five different mathematical models:

- I. Gordon-Taylor including the components' densities and T_g [145,146]
- II. Gordon-Taylor including the factor k based on the linearisation of measured values [74]
- III. Gordon-Taylor including the factor k based on a least-squares fitting to measured values
- IV. Kwei including the components' densities and T_g and a factor q obtained by a least-squares fitting to measured values [147,148]
- V. Couchman-Karaszi including the components' specific heat capacities and T_g [149,150]

For the Gordon-Taylor approach, the glass transition temperature of the ASDs or in general of the mixed system, $T_{g,mix}$, can be calculated by Eq. (3.1), where T_{g1} and T_{g2} represent the glass transition temperatures of drug and polymer, respectively. The variable x gives the drug content in the system in weight-by-weight. The constant k can be obtained by Eq. (3.2), which utilises the true densities ρ_1 and ρ_2 as well as glass transition temperatures of the components. An alternative approach is the linearisation of an experimental data set. By transforming Eq. (3.1) to Eq. (3.3), the term $T_{g,mix} - T_{g1}$

can be plotted against $(1-x)/x$. The slope of this plot divided by $T_{g2} - T_{g,mix}$ is the constant k , which can be calculated for each data point to find the mean value. The third option to determine k was by least-square fitting to experimentally measured values. This was done with the Solver-function in Microsoft Excel.

$$T_{g,mix} = \frac{x \cdot T_{g1} + k \cdot (1-x) \cdot T_{g2}}{x + k \cdot (1-x)} \quad (3.1)$$

$$k = \frac{T_{g1} \cdot \rho_1}{T_{g2} \cdot \rho_2} \quad (3.2)$$

$$T_{g,mix} - T_{g1} = k \cdot (T_{g2} - T_{g,mix}) \cdot \frac{1-x}{x} \quad (3.3)$$

$T_{g,mix}$ = glass transition temperature of mixed system; T_{g1} = glass transition temperature of the drug; T_{g2} = glass transition temperature of the polymer; k = constant; x = drug load (w/w); ρ_1 = true density of the drug; ρ_2 = true density of the polymer

The different models as well as different systems were compared using the area under the curve (AUC) of the respective function. Integration of the Gordon-Taylor formula leads to Eq. (3.4). The integration constant C was determined for $F(0) = 0$ after rearranging the primitive function according to Eq. (3.5) and Eq. (3.6). With the obtained C , the area under the curve from zero to 100% drug content was calculated with Eq. (3.7), where the second part can be neglected as $F(0) = 0$ per definition of C , as described earlier.

$$F(x) := \int T_{g,mix}(x) dx = \frac{[(T_{g2} - T_{g1}) \cdot k] \cdot \ln(x - k \cdot x + k) + [x \cdot (T_{g1} - k \cdot T_{g2} - k \cdot T_{g1} + k^2 \cdot T_{g2})]}{(1-k)^2} + C \quad (3.4)$$

$$F(0) = \frac{[(T_{g2} - T_{g1}) \cdot k] \cdot \ln(k)}{(1-k)^2} + C = 0 \quad (3.5)$$

$$C = \frac{-[(T_{g2} - T_{g1}) \cdot k] \cdot \ln(k)}{(1-k)^2} \quad (3.6)$$

$$F(1) - F(0) = \frac{T_{g1} - k \cdot T_{g2} - k \cdot T_{g1} + k^2 \cdot T_{g2}}{(1-k)^2} + C \quad (3.7)$$

C = integration constant

The Kwei model [147,148] (Eq. (3.8)) and the Couchman-Karasz model [149,150] (Eq. (3.9)) were used for comparison. For Kwei, an additional interaction parameter q is introduced which is determined by least squares fitting. For k , the constant based on the components' densities was used (Eq. (3.2)). Couchman-Karasz is similar to Gordon-Taylor including the specific heat capacities of drug and polymer, c_1 and c_2 , in the calculation instead of the densities.

$$T_{g,mix} = \frac{x \cdot T_{g1} + k \cdot (1-x) \cdot T_{g2}}{x + k \cdot (1-x)} + q \cdot (x - x^2) \quad (3.8)$$

$$T_{g,mix} = \frac{x \cdot T_{g1} \cdot c_1 + (1-x) \cdot T_{g2} \cdot c_2}{x \cdot c_1 + (1-x) \cdot c_2} \quad (3.9)$$

q = Kwei interaction parameter; c_1 = specific heat capacity of the drug; c_2 = specific heat capacity of the polymer

The solubility curves were calculated using the Kyeremateng and the Flory-Huggins model. Kyeremateng [151] follows Eq. (3.10), where $T_{s,mix}$ is the solubility temperature of the drug in the polymer, T_m is the melting temperature of the drug, x is the drug content in weight-by-weight and A , b and E are fitting parameters. According to literature, it is recommended to set $b = 0.05$ and only vary A and E following a least-squares fitting [30].

$$T_{s,mix} = -A \cdot e^{-100 \cdot b \cdot x} + T_m + E \quad (3.10)$$

$T_{s,mix}$ = solubility temperature of the drug; A , b and E = fitting parameters; T_m = melting temperature of the drug

For the Flory-Huggins equation [32,40] (Eq. (3.11)), the interaction parameter χ was determined by transforming the formula to Eq. (3.12), so one can plot the left side of the equation against $(1-\phi)^2$ with experimental values. The slope equals the interaction parameter χ [133]. With χ , the curve was calculated over the whole range between zero and 100% drug load. Additional parameters in the Flory-Huggins model include the molecular weight of drug and polymer, M_{w1} and M_{w2} , the gas constant R and the molar melting enthalpy of the drug, Δh_m . The volume fraction of the drug, ϕ , was calculated by Eq. (3.13) from the drug content x in w/w and the true densities of drug and polymer, ρ_1 and ρ_2 , respectively.

$$T_{s,mix} = \frac{1}{\left\{ \left(1 - \frac{M_{w1} \cdot \rho_2}{M_{w2} \cdot \rho_1} \right) \cdot (1-\phi) \right\} + \ln \phi + (1-\phi)^2 \cdot \chi} \cdot \left(\frac{-R}{\Delta h_m} + \frac{1}{T_m} \right) \quad (3.11)$$

$$\left(\frac{1}{T_{s,mix}} - \frac{1}{T_m} \right) \cdot \frac{-\Delta h_m}{R} - \left(1 - \frac{M_{w1} \cdot \rho_2}{M_{w2} \cdot \rho_1} \right) \cdot (1-\phi) - \ln \phi = \chi \cdot (1-\phi)^2 \quad (3.12)$$

$$\phi = \frac{\frac{x}{\rho_1}}{\frac{x}{\rho_1} + \frac{1-x}{\rho_2}} = \frac{x \cdot \rho_2}{x \cdot \rho_2 + (1-x) \cdot \rho_1} \quad (3.13)$$

M_{w1} = molecular weight of the drug; M_{w2} = molecular weight of the polymer; ϕ = volume fraction of the drug; R = gas constant; Δh_m = molar melting enthalpy of the drug; χ = interaction parameter

After assessment of the suitability of the single models, it was decided not to calculate all the options for trimethoprim ASDs. Here, it was focused on the Gordon-Taylor equation with fitted parameter k as described in Eq. (3.1). Solubility curves were based on the Flory-Huggins model (see Eq. (3.11) to (3.13)).

3.2.6.2 1H-NMR parameters

To determine the maximum possible shift difference in ppm, δ_{max} , as well as the dissociation constant K_D in mol/L, three mathematical models, originally from ligand-protein binding analysis, were applied to linearise the data sets [88,89]. These include a Scot-plot (Eq. (3.14)), a Scatchard-plot (Eq. (3.15)), and a Benesi-Hildebrand-plot (Eq. (3.16)). For all of them, the total ligand concentration in mol/L, $[L]$, as well as the observed peak shift difference in ppm, δ_{obs} , were needed from the single measurements. The observed peak shift difference refers to the difference between the data point and the minimum drug:polymer ratio, being the maximum observed peak shift. The

maximum possible shift difference refers to a theoretical complete complex formation of drug and polymer. It is important to mention that the here used K_D cannot be equalised with a dissociation constant in protein binding studies due to the larger number and high variance in possible binding sites.

$$\frac{[L]}{\delta_{\text{obs}}} = \frac{[L]}{\delta_{\text{max}}} + \frac{K_D}{\delta_{\text{max}}} \quad (3.14)$$

$$\frac{\delta_{\text{obs}}}{[L]} = \frac{-\delta_{\text{obs}}}{K_D} + \frac{\delta_{\text{max}}}{K_D} \quad (3.15)$$

$$\frac{1}{\delta_{\text{obs}}} = \frac{K_D}{\delta_{\text{max}} \cdot [L]} + \frac{1}{\delta_{\text{max}}} \quad (3.16)$$

δ_{max} = maximum possible peak shift; δ_{obs} = observed peak shift; $[L]$ = ligand concentration; K_D = dissociation constant

3.2.7 Molecular modelling

3.2.7.1 Molecular dynamics simulations

Molecular dynamics simulations as well as quantum mechanics calculations were performed with the Schrödinger MS Maestro software suite (version 13.9.138, MMshare Version 6.5.138, release 2024-1 on the Linux-x86_64 platform, Schrödinger, USA). The generation of small molecules was initiated through the utilisation of the drawing panel, followed by a conformational search that employed the OPLS4 force field. The experimental conditions comprised a low-mode sampling with a maximum number of steps set at 1,000 and 100 steps per rotatable bond, under the assumption of a solvent-free surrounding. The creation of polymers was achieved through the polymer builder function, which facilitated the drawing and combination of monomers. These monomers were completed by initiator and terminator units, which were incorporated into the system.

Table 3.8 presents the composition of the polymers. The monomer count was fixed to obtain polymer chains with a molecular mass that was one-tenth of the actual mass, thus ensuring that the molecule sizes and atom numbers were manageable in the calculations. For MAE-A, a factor of one twentieth was selected because of the elevated molecular mass, while no factor for downsizing was deemed necessary for HPMCAS. Except for HPMCAS and HPMC, all multi-component polymers were formed as random copolymers. In this instance, the number of monomers corresponds to the total number of different monomers represented as a given percentage.

From small molecules and/or polymers, cells with a given number of molecules were formed by the disordered system builder (see Table 3.9). The choice of periodic boundary conditions was made to be orthorhombic, and the initial state was defined as the mode “tangled chain”, which is a non-native, unfolded, randomly coiled conformation. This configuration is not energetically favourable and serves as a starting point for simulations, which realistically lead to a more energetically stable state. The number of molecules per cell was selected to ensure the systems had comparable masses, given that formulations analysed in a laboratory setting are also scaled according to masses and not

atom or molecule count. The required number of molecules was calculated for a cell with 10%, 30%, 50%, 70%, 90% and 100% drug load by dividing the respective molecular mass by the weight-percent content and multiplying it by a factor of 10,000/3. This resulted in cells with total atom numbers of 40,000-50,000. The factor is exclusively employed for scaling to meaningful cell sizes and possesses no underlying physical basis. Additionally, a simulation was conducted for each polymer, with a single polymer molecule and 500 API molecules utilised in this process.

Table 3.8 Parameters for the used polymers in molecular modelling. Based on the actual molecular weight, a downsizing factor was derived to achieve comparable molecular weights in the cells.

Polymer	Actual molecular weight [g/mol]	Used molecular weight in molecular dynamics [g/mol]	Downsizing factor	Number of monomers per molecule
MAE-A	320,000	16,000	20	186
EPO	47,000	4,700	10	40
HPMCAS	17,000	17,000	1	78
HPMC	84,000	8,400	10	38
VA64	57,500	5,800	10	57
K30	49,000	4,900	10	44
PAA	100,000	10,000	10	138
PSSA	75,000	7,500	10	41

Table 3.9 Number of molecules per cell at different drug loads of either cinnarizine or trimethoprim.

API content [% w/w]	Number of molecules per cell									
	CIN	TMP	MAE-A	EPO	HPMCAS	HPMC	VA64	K30	PAA	PSSA
0			21	71	20	40	57	68	33	44
10	91	115	19	64	18	36	52	61	30	40
30	272	345	15	50	14	28	40	48	23	31
50	453	575	10	35	10	20	29	34	17	22
70	634	805	6	21	6	12	17	20	10	13
90	815	1034	2	7	2	4	6	7	3	4
100	906	1149								

Subsequently, a molecular dynamics simulation was performed, encompassing a compressive relaxation protocol of seven stages. The simulation itself was conducted over a total period of 20 nanoseconds, with a recording interval of 200 picoseconds per trajectory in the NPT ensemble (constant number of atoms, pressure, and temperature) at 300.0 K and 1.01325 bar. When evaluating the number of molecular interactions of the resulting simulations, the maximum distances for the single types were as follows: 2.8 Å for aliphatic and aromatic H-bonds, 5.0 Å for salt bridges, 6.6 Å for π -cation interactions, 4.4 Å and 5.5 Å for face to face and edge to face π - π interactions, respectively. Besides defined interaction types, the quantitation of beneficial contacts was facilitated through the implementation of cut-off ratios of 1.3 (with hydrogen bonds and salt bridges excluded), whereas bad and ugly contacts were defined with cut-off ratios of 0.89 and 0.75, respectively. The classification is based on the geometric and energetic quality of atom-atom contacts and their

proximity. With more bad or ugly contacts, the atoms are closer or even too close to each other to deliver a stable system. The cut-off ratio is calculated by dividing the interatomic distance by the sum of the van der Waals radii of the two atoms.

3.2.7.2 Solubility parameters

When creating a cell of only one component, for either the active or the polymer, the simulation can also be evaluated on the resulting solubility parameters of the substance in question. The Hildebrand solubility parameter (δ_{total}) was calculated from the total cohesive energy density via molecular dynamics. While the software partitions the output into van der Waals and electrostatic terms, these values represent the specific energetic contributions to the total cohesive energy rather than a formal Hansen three-component analysis. By summing these potential energy terms, a total Hildebrand value is obtained. The van der Waals solubility parameter δ_{vdW} includes dispersive non-covalent interactions as London forces or induced dipoles. The electrostatic solubility parameter δ_e comprises Coulomb interactions as permanent dipoles and ionic bonds. The overall solubility parameter δ_{total} can be derived by using Eq. (3.17). The solubility parameters are expressed in $\text{MPa}^{1/2}$.

$$\delta_{total} = \sqrt{\delta_{vdW}^2 + \delta_e^2} \quad (3.17)$$

δ_{total} = overall solubility parameter (Hildebrand); δ_{vdW} = van der Waals solubility parameter; δ_e = electrostatic solubility parameter

Substances are assumed to be miscible or dissolvable in each other when $\Delta\delta_{total}$ is below $2 \text{ MPa}^{1/2}$, with higher $\Delta\delta_{total}$ values implying poor compatibility in a proportional manner.

3.2.7.3 pK_a -estimation by quantum mechanics

For the single substances, an estimated pK_a -value could be obtained by molecular quantum mechanics via the Jaguar quantum mechanics engine in the Schrödinger MS Maestro software. In case of small molecules, the entire molecule was represented; however, for polymers, it was decided to reduce the molecules to their monomers to simplify the process. Alongside the calculation, a conformational search was performed with an energy window of 12.0 kcal/mol. Water was chosen as the solvent in the geometry optimisation step. In the case of acids, a proton was selected for splitting off, while in the case of bases, an oxygen or nitrogen atom was chosen as the acceptor for the proton.

4 Results and discussion

4.1 Material properties

The raw materials were examined to determine their degradation temperature as well as their glass transition or melting temperature. Additionally, the densities of the solid powders were measured using helium pycnometry. A summary of the results is provided in Table 4.1.

Table 4.1 Thermal properties and true densities of the raw materials.

Substance	Mean molecular weight [g/mol]	Degradation [°C]	Glass transition [°C]	Melting point [°C]	True density [g/cm ³]
CIN	368.51	271.2 ± 5.8	7.8 ± 0.2	119.6 ± 0.5	1.14 ± 0.01
TMP	290.32	271.5 ± 13.9	59.3 ± 0.5	201.6 ± 0.5	1.29 ± 0.01
MAE-A	320,000	190.6 ± 2.3	115.1 ± 1.5		1.20 ± 0.03
EPO	47,000	223.3 ± 5.8	45.1 ± 0.5		1.18 ± 0.05
HPMCAS	17,000	277.0 ± 11.9	119.5 ± 0.7		1.27 ± 0.01
HPMC	84,000	286.7 ± 1.6	101.5 ± 2.2		1.47 ± 0.16
VA64	57,500	253.4 ± 1.4	107.4 ± 0.6		1.05 ± 0.01
K30	49,000	169.0 ± 1.1	161.8 ± 0.8		1.01 ± 0.00

Solubility parameters were calculated for dispersive interactions and for Coulomb interactions. An overall solubility parameter was calculated using Eq. (3.17). The results are summarised in Table 4.2. The results of the pK_a-calculations are summarised in Table 4.3. Figure 3.1 in chapter 3.1.1 shows the molecular structures of both active substances, indicating the potential protonation positions.

Table 4.2 Solubility parameters for the active substances and polymers used in this study.

	overall solubility parameter δ_{total} [MPa ^{1/2}]	van der Waals solubility parameter δ_{vdW} [MPa ^{1/2}]	Electrostatic solubility parameter δ_e [MPa ^{1/2}]
Cinnarizine	19.14	18.10	6.22
Trimethoprim	25.21	18.80	16.79
MAE-A	14.31	10.77	9.41
EPO	13.03	12.19	4.62
HPMCAS	17.44	12.87	11.76
HPMC	17.69	13.11	11.89
VA64	14.94	13.60	6.17
K30	15.21	13.81	6.37
PAA	19.88	12.83	15.19
PSSA	18.95	12.74	14.02

A single pK_a value is provided for the polymers under investigation, as the analysis was based on their monomeric units in order to simplify the method. However, the acid-base behaviour of the polymers is substantially more complex. Among the polymers, PSSA is the strongest acid with a negative pK_a value, while PAA is also strongly acidic. In accordance with assumptions, MAE-A

exhibited a slightly higher degree of acidity in comparison to HPMCAS, while EPO demonstrated characteristics of a weak base. Regarding the APIs, it is notable that each molecule contains two nitrogen atoms as protonation sites.

Table 4.3 Results of the pK_a calculations using Jaguar.

	CIN	TMP	MAE-A	HPMCAS	EPO	PAA	PSSA
$pK_{a,1}$	6.39	4.11	4.44	5.03	7.63	3.32	-0.91
$pK_{a,2}$	6.40	5.86					
$pK_{a,1}$ for a protonated pos. 2	2.13	-1.90					
$pK_{a,2}$ for a protonated pos. 1	4.33	0.55					

4.2 Cinnarizine

Essential contents of this chapter are based on the research article manuscript “On the influence of polymers in stabilising and enhancing the performance of cinnarizine amorphous solid dispersions”, by Selina Schmidt, Jörg Breitzkreutz and Heiko A. Schiffter; Target Journal: Next Research (2026), which has been prepared and submitted to the target journal.

4.2.1 Melt quenching and hot melt extrusion

ASDs were prepared by melt quenching inside the DSC. Binary physical mixtures of cinnarizine and the different polymers were heated, rapidly cooled with a cooling rate of 20 K/min, and reheated to detect glass transitions, crystallisation, and melting events. Heating above the melting point of the active ingredient and subsequent cooling led to the formation of amorphous solid dispersions, whose mixed glass transition temperatures could then be determined as well as the amount of residual crystallinity.

For all six polymers, it was possible to form a homogeneous amorphous system with drug loads of 10% (w/w) and 30% (w/w). At a drug load of 50% (w/w), only HPMCAS was able to form a completely amorphous system. At drug loads of 70%, 80% and 90% (w/w), residual crystallinity was found in all formulations (see Figure 4.1). The values represent how much of the incorporated drug was in a crystalline state after melting and subsequent rapid cooling. The crystallinity was shown by either a single melting endotherm in the reheating cycle, or by both a recrystallisation (cold-crystallisation) and melting signal upon reheating. In the latter case, the resulting enthalpies were offset against each other in order to consider only the surplus in melting.

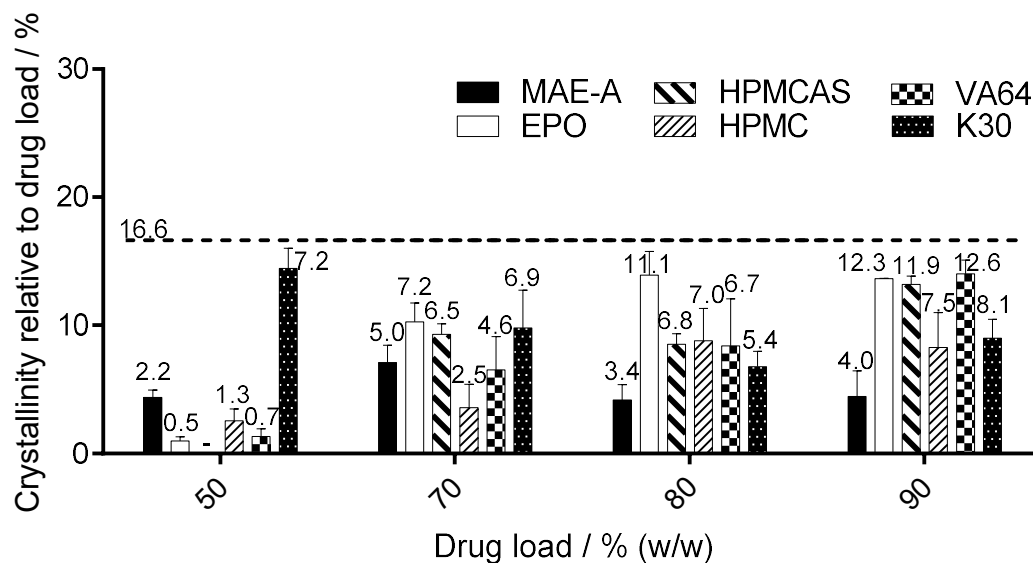


Figure 4.1 Residual cinnarizine crystallinity of the amorphous solid dispersions after preparation by melt quenching in the DSC. Given is the degree of crystallinity relative to the drug load of 50%, 70%, 80%, and 90% (w/w) calculated using the melting enthalpy. The values represent the mean $\pm$ standard deviation ($n = 3$). The dashed line shows the residual crystallinity of cinnarizine after melt quenching. The numbers above the bars give the degree of crystallinity relative to the whole system (mean value of $n = 3$).

The residual crystallinity for pure cinnarizine was 16.6%. Polymer addition reduced this value and increased the amount of amorphous drug. A completely amorphous system could be obtained for HPMCAS formulations with 50% (w/w) cinnarizine load. However, at higher drug loads HPMCAS did not outperform the other polymers tested. For MAE-A, the residual crystallinity was almost constant regardless of the drug load. For EPO, HPMC and VA64, the crystallinity increased with increasing cinnarizine content, both concerning the crystallinity relative to the drug load and relative to the whole system. For K30, an increase in drug load surprisingly resulted in a decrease in relative crystallinity of the prepared systems.

In HME, drug loads of 10% and 30% (w/w) in a completely amorphous state were successfully obtained for all six polymers. Figure 4.2 and Figure 4.3 illustrate that 50% (w/w) drug load was attainable with both MAE-A and HPMCAS. The input of mechanical shear forces, alongside heating, enhanced drug embedding into the polymer matrix in the MAE-A experiments compared to using DSC alone. For HPMC and K30, the differences in residual crystallinity after melt quenching and HME were non-significant ($p = 0.37$ and 0.50 , respectively). For EPO and VA64, the residual crystallinity was significantly higher when prepared by HME (both $p < 0.05$). Despite the extended heating time compared to the DSC method, along with the additional mixing and kneading facilitated by mechanical forces, the process did not positively influence the degree of crystallinity. The time interval between extrusion and DSC measurement was kept as short as possible, although the potential for recrystallisation during this period remained. This lag-time between the formation of the system and the measurement does not exist when preparing amorphous solid dispersions in the

DSC with immediate reheating for subsequent analysis. Furthermore, cooling in the DSC occurs much faster than in hot melt extrusion.

The preparation of ternary systems was achieved through the utilisation of melt quenching and hot melt extrusion. In both preparation methods, the residual crystallinity was higher compared to the respective single polymer systems. For MAE-A/VA64 ASDs, the crystallinity was found to be higher than that of cinnarizine drug substance after extrusion. In both combinations MAE-A/VA64 and HPMCAS/VA64, it was found that more amorphous cinnarizine could be embedded by melt quenching compared to extrusion. The embedding capability does not benefit from the mechanical input in this instance, but the time interval between melting in the extruder and subsequent DSC analysis leads to instabilities and crystallisation. Section 4.2.4 describes the long-term storage of melt extruded, binary ASDs, where it was found that an existing residual crystallinity tended to continue to grow during storage. Depending on the system's stability, this can happen in a short time period, as seen in the pure cinnarizine samples that crystallised visibly when exiting the extruder. An additional factor is the comparatively slower cooling speed in contrast to that of melt quenching. Systems employing VA64 alone demonstrate equivalent behaviour. Systems with MAE-A alone benefit from the mechanical input due to the high melt viscosity of the polymer. However, in the context of HPMCAS/VA64 in hot melt extrusion, a reduction in crystallinity was observed in comparison to VA64 alone. Conversely, when HPMCAS was employed as the sole polymer, the entire drug load could be embedded within an amorphous state. Consequently, the ternary combination is also rendered ineffective in this instance. Despite the larger degree of residual crystallinity in the samples, only one T_g was detected in all cases, indicating a homogeneous mixture of the polymers.

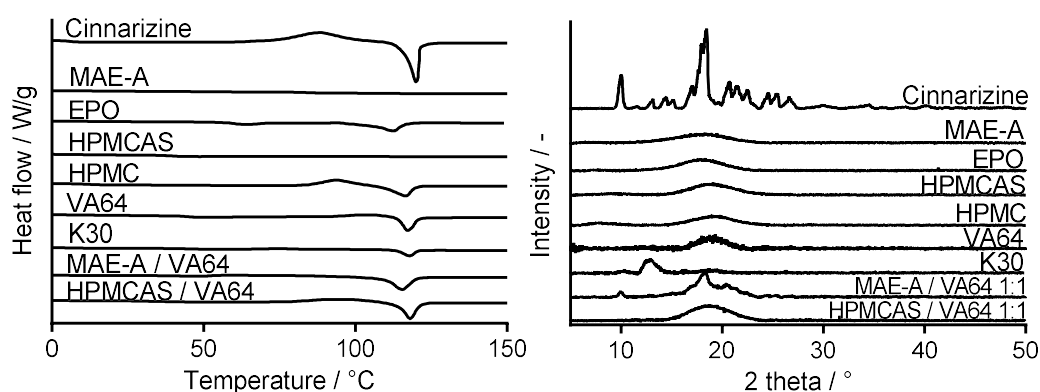


Figure 4.2 DSC (left) and XRPD (right) plots of binary drug-polymer formulations prepared by hot melt extrusion. The cinnarizine and the cinnarizine-polymer systems with 50% (w/w) drug load are displayed. Datasets are stacked on the y-axis for better visualisation; exothermal events are plotted upwards in the DSC diagrams.

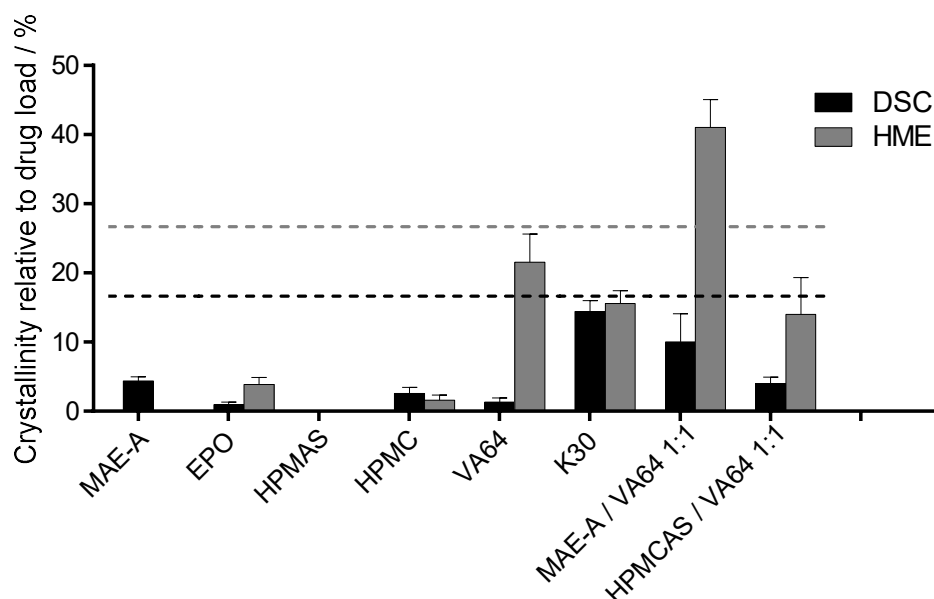


Figure 4.3 Comparison of the residual crystallinity of the amorphous solid dispersions with 50% (w/w) cinnarizine drug load prepared by melt quenching in the DSC (DSC) and hot melt extrusion (HME). The degree of crystallinity relative to the drug load is displayed as mean $\pm$ standard deviation ($n = 3$). Black bars show the melt-quenched and grey bars show the extruded samples. The dashed black line and grey line show the residual crystallinity of cinnarizine after melt quenching and hot melt extrusion, respectively.

The residual crystallinity of the samples with 50% (w/w) drug load prepared by melt quenching increased in the sequence: HPMCAS < EPO < VA64 < HPMC < MAE-A < K30. For ASDs made via melt extrusion, the ranking for residual crystallinity at 50% (w/w) drug load was HPMCAS = MAE-A < HPMC < EPO < K30 < VA64.

It was expected that polymers with alkaline functional groups would not stabilise the amorphous state of the weak base cinnarizine as good as polymers with acidic functional groups. The study's findings provided unexpected results regarding the performance of certain polymers. The performance of EPO in melt quenched systems surpassed expectations, while MAE-A was surprisingly less effective in melt quenching. The melt viscosity of MAE-A is highest compared to the other polymers (above 10,000 Pa·s [152], compared to 2,000-3,000 Pa·s for HPMCAS [153,154]), making the additional input of mechanical shear forces a requirement for proper mixing and dissolution of cinnarizine in the polymer glass. In hot melt extrusion, MAE-A and HPMCAS performed best, aligning with what was anticipated, meaning the superior performance of acidic polymers. However, the poor performance of VA64 and K30 came as a surprise. It was expected that the hydrophobic domains could stabilise the lipophilic drug substance cinnarizine to a larger extent. In melt quenching, crystallinity was significantly lower in VA64-samples compared to K30 samples at 50% drug load. The values converged with increasing drug load, as polymer effects became less pronounced. VA64 comprises hydrophobic vinyl acetate groups in addition to povidone's vinyl pyrrolidone groups. The increased hydrophobicity in VA64 helps to stabilise cinnarizine in its amorphous state, supporting the hypothesis of a beneficial effect of hydrophobic domains. Still, the

effect does not surpass the one from acidic, polar polymers. From literature, it was known that both povidone and copovidone can form stable ASDs with weakly basic compounds [62,74]. However, both polymers tend to ab- and adsorb water from their environment due to their high hygroscopicity, leading to instabilities [36,155]. Residual crystallinity in EPO systems after hot melt extrusion was again lower than expected. As EPO contains basic structures, it should not be suitable for stabilising basic drug candidates. Besides the basic dimethylaminoethyl methacrylate monomer, EPO consists of butyl methacrylate and methyl methacrylate (see Figure 3.2). Both monomers form a hydrophobic domain, which is beneficial in incorporating cinnarizine as discussed above for K30 and VA64. When counting the butyl and the methyl methacrylate as one type of hydrophobic monomers, the ratio compared to the basic dimethylaminoethyl methacrylate is 1:1. The high number of hydrophobic moieties helps to stabilise the amorphous lipophilic drug.

Furthermore, EPO has a low glass transition temperature, and this characteristic was expected to lead to more instabilities, as the mixed T_g at 50% (w/w) drug load was lower compared to that of other systems, resulting in higher molecular mobility at room temperature. In contrast, the low T_g of EPO offers the advantage of improved softening and enhanced incorporation and mixing in HME due to reduced viscosity. During extrusion, polymers with lower T_g are more mobile, which facilitates better molecular mixing and dispersion of the drug, often resulting in lower initial residual crystallinity. The same molecular mobility that aids mixing can also allow the amorphous drug to reorganise and crystallise more readily when exposed to environmental stress during storage. In contrast, high- T_g polymers are more rigid during extrusion, which may lead to higher residual crystallinity immediately after HME if the mechanical input is not strong enough, but provide stronger kinetic stabilisation of the amorphous drug over time. The polymer with the highest T_g used in this study is K30, which led to the highest crystallinity after melt quenching without mechanical input, supporting this hypothesis.

The complete data on DSC- and XRPD-plots as well as numerical values for the residual crystallinities in table format are listed in the appendix (Figure 8.14, Figure 8.16, Figure 8.24 - Figure 8.29 and Table 8.4).

4.2.2 Spray drying

Systems with MAE-A (50% and 70% drug load (w/w)), HPMCAS (30% and 50% drug load (w/w)), HPMC (30% and 50% drug load (w/w)), VA64 (10%, 30%, 50% and 70% drug load (w/w)), K30 (30% and 50% drug load (w/w)), Soluplus (30% drug load (w/w)) and a 1:1 mixture of MAE-A and VA64 (50% drug load (w/w)) were prepared by spray drying. Cinnarizine alone could not be spray-dried due to its low glass transition temperature and its sticking to the glass parts of the spray dryer, especially in the cyclone. The same was true for formulations with cinnarizine and EPO.

None of the formulated samples was completely amorphous. The residual crystallinities are summarised in Figure 4.4.

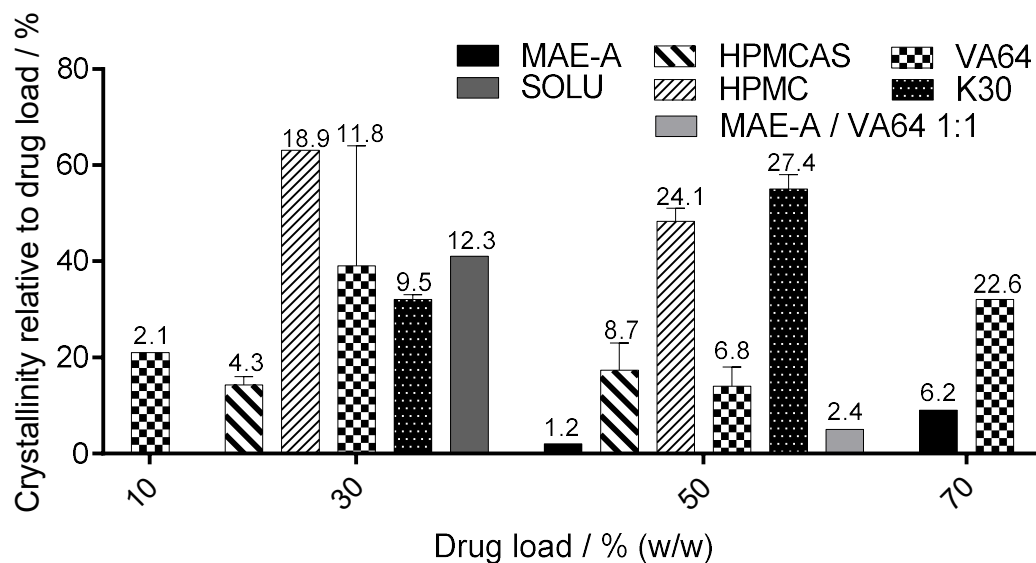


Figure 4.4 Residual cinnarizine crystallinity in the amorphous solid dispersions after spray drying. Given is the degree of crystallinity relative to the drug load of 10%, 30%, 50%, and 70% (w/w) calculated by the melting enthalpy. The values represent the mean $\pm$ standard deviation ($n = 3$). The numbers above the bars give the degree of crystallinity relative to the whole system (mean value of $n = 3$).

For VA64, all drug loads were tested. The crystallinity fluctuated and no clear trend was observable. For HPMCAS, HPMC and K30, which were tested at 30% and 50% drug load, crystallinity increased with a higher cinnarizine concentration. This fits to the observations made in hot melt extrusion and melt quenching. Crystallinity was lowest for systems with MAE-A with only 2.3% of the drug being crystalline at 50% drug load (equivalent to 1.2% of the whole system). With the combination of MAE-A and VA64, crystallinity was twice as high as for MAE-A alone but significantly reduced compared to VA64 alone. Still, if an addition of VA64 is not required, the formulation's stability does not benefit from the ternary system. Regarding the manufacturing method, the ternary system made in spray drying outperformed the equivalent one from hot melt extrusion and melt quenching. The dissolution of the polymers in an organic solvent seem to add a benefit in the system structure, homogeneity and stability; here, the mixing of the system in solution was superior compared to the one obtained by melting and mechanical forces, attributable to the high melt viscosity of MAE-A as discussed previously. For the other polymers, crystallinity was lower after melt extrusion compared to spray drying. Longer residence times at elevated temperatures and residual solvents enhance nucleation processes leading to large-scale crystallisation [156].

The complete data on DSC- and XRPD-plots as well as numerical values for the residual crystallinities in table format are listed in the appendix (Figure 8.21, Figure 8.22 and Table 8.4).

4.2.3 Content determination

The cinnarizine content of the amorphous solid dispersions prepared by hot melt extrusion deviated from the targeted drug loads in some cases, with underestimations being more frequent than

overestimations. Thus, all subsequent calculations involving the drug content were based on the analytically determined actual content. The results are presented in Table 4.4. The observed deviations, particularly in the case of the HPMCAS and HPMC samples, could be attributed to the behaviour of the powder during the extrusion process. While adhesion to the screws and to the inside of the barrel was an issue for some formulations, cinnarizine mixtures with cellulose derivatives adhered even more strongly. This could also have resulted in segregation. Repeating the extrusion process led to comparable results. For this reason, the samples with the reduced drug contents were used for the subsequent stability tests.

Table 4.4 Cinnarizine content in amorphous solid dispersions prepared by hot melt extrusion. The values represent the mean actual content $\pm$ standard deviation ($n = 3$). The relative standard deviation is given in brackets.

Polymer	Actual content		
	Target 10%	Target 30%	Target 50%
MAE-A	11.5 $\pm$ 0.1% (1.1%)	31.1 $\pm$ 0.9% (2.8%)	50.5 $\pm$ 0.3% (0.7%)
EPO	8.6 $\pm$ 0.4% (4.5%)	26.9 $\pm$ 0.6% (2.3%)	46.2 $\pm$ 1.8% (3.8%)
HPMCAS	6.5 $\pm$ 1.4% (21.5%)	25.6 $\pm$ 1.7% (6.8%)	42.9 $\pm$ 2.9% (6.7%)
HPMC	6.8 $\pm$ 0.9% (13.0%)	24.4 $\pm$ 0.8% (3.2%)	41.1 $\pm$ 0.1% (0.3%)
VA64	9.3 $\pm$ 0.0 % (0.3%)	29.0 $\pm$ 0.5% (1.9%)	49.4 $\pm$ 0.7% (1.4%)
K30	8.7 $\pm$ 0.0% (0.1%)	26.8 $\pm$ 0.6% (2.1%)	48.6 $\pm$ 1.4% (2.9%)
VA64/MAE-A 1:1	-/-	-/-	51.3 $\pm$ 1.6% (3.0%)
VA64/HPMCAS 1:1	-/-	-/-	53.8 $\pm$ 0.1% (0.2%)

Samples prepared by spray drying matched the targeted content better, though no samples with 10% drug load were prepared. For samples with 30% and 50% drug load, the determined contents were slightly above the target. Only one sample was prepared with 70% drug load, where 67% were found, which is also acceptable.

Table 4.5 Cinnarizine content in amorphous solid dispersions prepared by spray drying. The values represent the mean actual content $\pm$ standard deviation ($n = 3$). The relative standard deviation is given in brackets.

Polymer	Actual content		
	Target 30%	Target 50%	Target 70%
MAE-A	-/-	53.4 $\pm$ 4.0% (7.5%)	67.0 $\pm$ 6.6% (9.9%)
HPMCAS	32.5 $\pm$ 2.4% (7.2%)	53.5 $\pm$ 1.4% (2.7%)	-/-
HPMC	32.8 $\pm$ 1.1% (3.4%)	55.0 $\pm$ 0.8% (1.5%)	-/-
VA64	32.9 $\pm$ 2.4% (7.3%)	49.8 $\pm$ 3.4% (6.9%)	-/-
K30	32.6 $\pm$ 1.1% (3.3%)	51.1 $\pm$ 3.2% (6.2%)	-/-
VA64/MAE-A 1:1	-/-	54.3 $\pm$ 3.7% (6.8%)	-/-

4.2.4 Long term stability of ASDs prepared by hot melt extrusion

The physical stability of the ASDs prepared by hot melt extrusion was assessed by examining their thermal behaviour via DSC and their X-ray diffraction patterns. Table 4.6 presents a summary of the results directly after manufacturing ($t = 0$) and following storage for three, six, nine, twelve, 18 and 24 months in the refrigerator (8 °C) and in climate chambers set at 25 °C / 60% relative

humidity and at 40 °C / 75% relative humidity. The DSC- and XRPD-plots are depicted in the appendix (Figure 8.24 - Figure 8.31).

When comparing the two analytical methods, it becomes evident that crystallinity was detected at a lower crystallinity level using DSC compared to XRPD. The XRPD technique requires a substantially higher degree of crystallinity to produce measurable results, whereas DSC can detect crystallinity even with small amounts. According to literature, the degree of crystallinity must be 5 - 10% to be recognisable by XRPD [82,83]. As illustrated in Table 4.6, the lowest degree of crystallinity detected by XRPD was 8.6% (in HPMC with 30% (w/w) cinnarizine load after three months of storage at 40 °C / 75% relative humidity). Some samples with higher degrees of crystallinity would have been classified as amorphous based on XRPD alone, reaching up to 15.2% (3 months refrigerator, K30). The sensitivity of the XRPD analysis is highly dependent on the chosen measurement settings. In this study, a relatively fast scan was performed, as XRPD results were used complementary to DSC analysis, which was the main method for crystallinity detection.

Examining stability reveals that mostly samples with initial crystallinity tend to undergo further crystallisation, while new crystallisation events were rare. ASDs with 30% (w/w) drug load in EPO, HPMC and VA64 crystallised during storage. Additionally, the sample comprising a 50% (w/w) drug load and HPMCAS exhibited crystallinity after two years of storage at 40 °C / 75% relative humidity. Harsher conditions did not always lead to less stable systems. For instance, VA64 samples with 30% (w/w) drug load showed crystallisation after 12 months of storage in both the refrigerator (8 °C) and at 25 °C / 60% relative humidity, but not at 40 °C / 75% relative humidity. Figure 4.5 shows the residual crystallinity over time. ASDs with MAE-A and HPMCAS are not included, as no crystallinity was found in MAE-A samples over the whole tested time of two years. HPMCAS-ASDs showed crystallinity only after two years at 40 °C / 75% relative humidity. Both polymers contain acidic functional groups, enabling increased molecular interactions with the weak base cinnarizine and thereby stabilising the amorphous state, as expected [57,62].

Similarly, when assessing the homogeneity of the amorphous systems, increased temperatures and humidity did not necessarily facilitate phase separation to a higher extend. HPMCAS samples with 10% and 50% (w/w) drug load showed two or more glass transitions after storage in the refrigerator, whereas those stored at 25 °C / 60% relative humidity were homogeneous. At 40 °C / 75% relative humidity, only the 50 % (w/w) drug load samples showed phase separation. Although there was a trend indicating increased phase separation with higher drug loads, no definitive correlation could be identified. In some cases, multiple glass transitions were detected at a 10% (w/w) loading but not at 30% (for HPMCAS and K30 in the refrigerator). In some cases, multiple phases were present in the starting material or at early stability time points but were not observed throughout the entire testing period.

Table 4.6 Physical stability of the melt extruded amorphous solid dispersions. The storage times are given in months. Numbers in red boxes indicate the degree of crystallinity relative to the whole system in percent as determined by the melting enthalpy in DSC (mean of $n = 3$). Stability studies with K30-ASDs were run for 18 months only, therefore the 24 months' time point is missing.

Polymer	Drug load [%]		25 °C / 60% r.h.						40 °C / 75% r.h.					
		0	3	6	9	12	18	24	3	6	9	12	18	24
MAE-A	10													
	30													
	50													
EPO	10								could not be measured, as formulations melted at 40 °C					
	30		0.7	1.6	1.8	1.4	1.7	2.2						
	50	1.8	29.4	27.5	28.7	23.3	24.2	23.2						
HPMCAS	10													
	30													
	50													9.6
HPMC	10													
	30							0.6	8.6	10.5	10.6	11.7	12.0	12.0
	50	1.5	9.8	15.4	18.7	18.8	18.9	18.5	33.8	33.4	30.1	32.6	30.8	27.9
VA64	10													
	30					0.1	0.6	0.7						
	50	12.3	16.4	18.0	15.2	15.9	16.2	15.3	17.9	28.6	30.8	31.3	28.6	29.5
K30	10													
	30													
	50	7.6	17.9	17.6	21.0	19.3	20.1		16.9	18.7	18.0	18.5	19.0	
Polymer	Drug load / %	Start	Refrigerator											
		0	3	6	9	12	18	24						
MAE-A	10													
	30													
	50													
EPO	10													
	30													
	50	1.8	2.1	4.3	3.3	2.1	7.5	2.2						
HPMCAS	10													
	30													
	50													
HPMC	10													
	30										0.2	0.9		
	50	1.5	1.2	0.3	3.3	3.3	18.4	2.7						
VA64	10													
	30								0.5	2.5	0.8			
	50	12.3	11.5	17.7	12.8	0.1	11.7	10.7						
K30	10													
	30													
	50	7.6	15.2	16.2	16.5	20.3	21.8							
fully amorphous (only one T_g)				amorphous-amorphous phase separation (two or more T_g s)					(partly) crystalline (melting peak detected)					
bold: XRPD-crystalline														

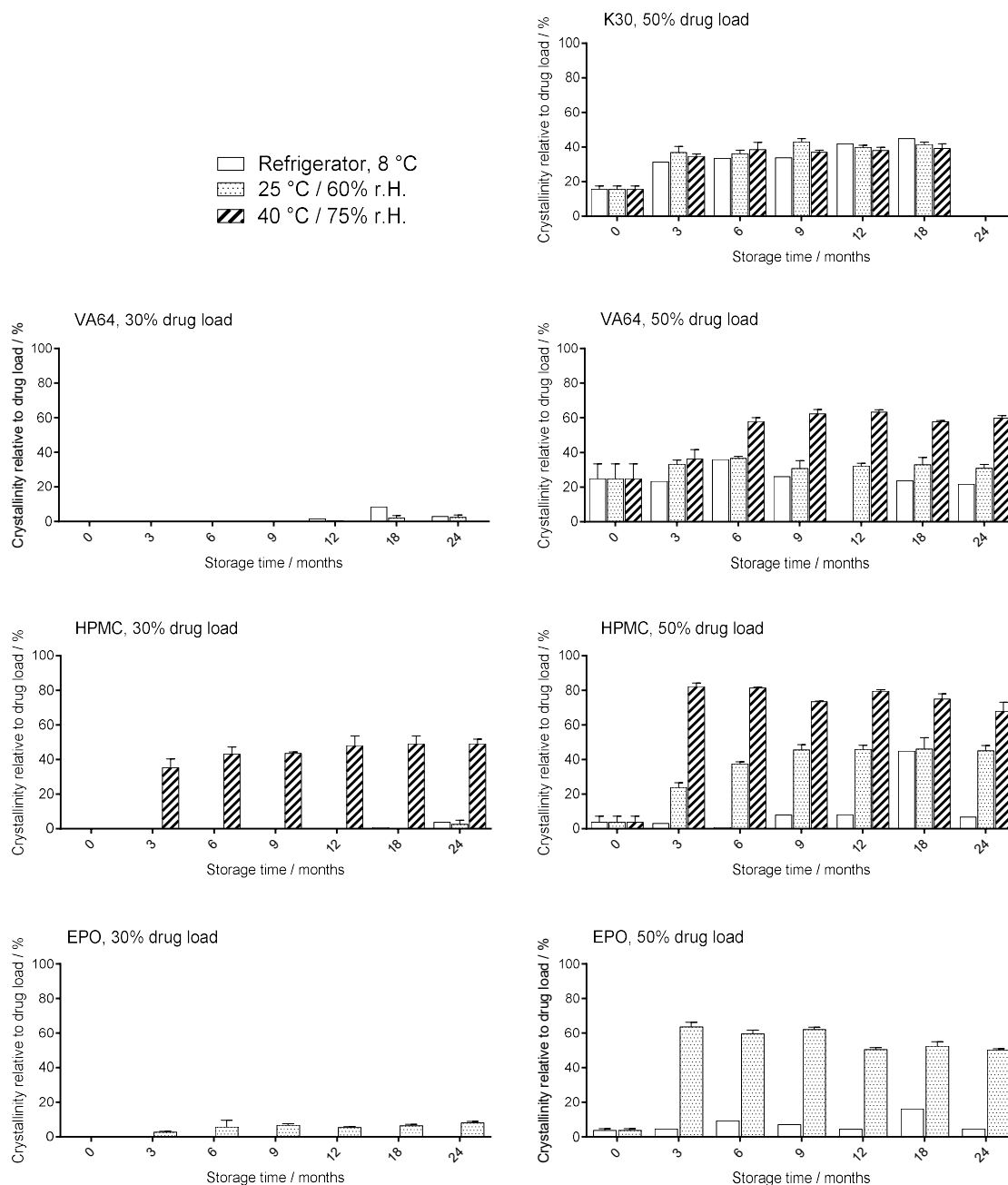


Figure 4.5 Degree of crystallinity relative to drug load during stability testing of samples prepared by hot melt extrusion. The plots show the crystallinity in samples with EPO, HPMC, VA64, and K30 measured via DSC (mean $\pm$ standard deviation, $n = 3$). Empty columns show the storage in the refrigerator (8 °C), dotted columns at 25 °C / 60% and hatched columns at 40 °C / 75% relative humidity. 30% drug load samples with K30 were all amorphous.

The vials containing the milled ASDs for stability testing were all sealed with a lid and Parafilm, having a low water vapour permeability. Still, the increased humidity in the climate chambers can penetrate the vials and lead to water sorption of the ASDs. The datasheet of the Parafilm supplier (Sigma Aldrich, USA) indicates a water vapour permeability of 0.88 g/m² per 24 hours at 38 °C and 90% relative humidity compared to storage with a desiccant. In the refrigerator, the relative humidity was not controlled, but measured with the result being 60 – 70%. The partly higher tendency for

phase separation in systems with MAE-A, HPMCAS, VA64, and K30 stored in the refrigerator compared to the other storage conditions might be linked to water condensation within the vials. The vials were sealed at ambient conditions in the laboratory and then stored without an additional drying or conditioning step. Entrapped water could condensate due to decreased temperatures, then accumulate on the surface of the milled ASDs, enabling sorption, and consequently, phase separation. Despite the presence of domains containing accumulated drug molecules, the low temperatures effectively prevented crystallisation.

4.2.5 Phase diagrams

Phase diagrams for binary systems of cinnarizine with MAE-A, EPO, HPMCAS, HPMC, VA64 and K30 were calculated. Figure 4.6 shows the results of the different models for HPMCAS and EPO as examples.

Examining the models for the glass transition curve in the HPMCAS system, it is evident that the theoretical Gordon-Taylor equation using the true densities of the components leads to an underestimation of the actual values (black solid line). The curve exhibits a pronounced negative deviation from the experimental values, resulting in a distinct area between both. The Gordon-Taylor curves for both the calculated and the fitted k -values demonstrate superior fit and only small discrepancies between them (black dashed and dotted lines). Additionally, the Couchman-Karasz curve depicts the experimental values to a similar extent (green line). In contrast, the Kwei model not only displays deviations in the form of a curve-shift but also exhibits a completely different shape (red line). For the solubility curves, all models align closely with the measured values but differ in their progression for the lower drug contents. In relation to EPO, all models, except for Couchman-Karasz, show an acceptable fit to the T_g values. The solubility curves again overlap for high drug contents yet display disparate shapes at lower levels.

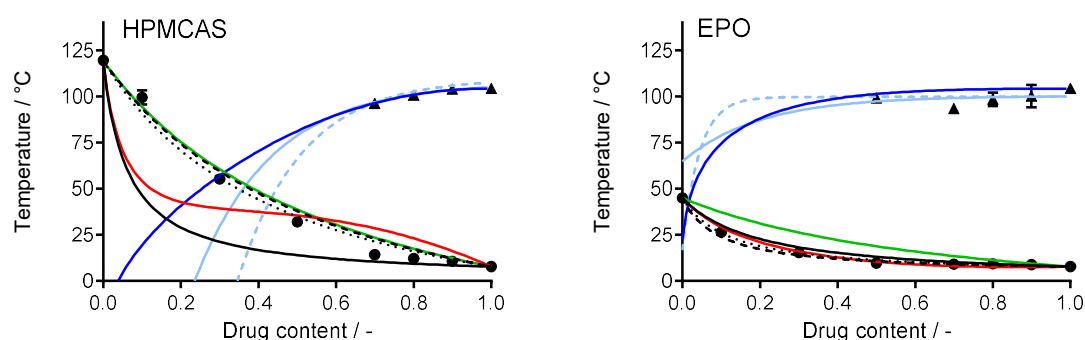


Figure 4.6 Phase diagrams of cinnarizine-HPMCAS and cinnarizine-EPO amorphous solid dispersions. Circles and triangles show the measured glass transition temperatures and solubility temperatures, respectively (mean $\pm$ standard deviation, $n = 3$). The lines represent the calculated curves for Gordon-Taylor (black; solid for theoretical k , dashed for calculated k , dotted for fitted k), Kwei (red), Couchman-Karasz (green), Flory-Huggins (dark blue) and Kyeremateng (light blue; solid for fixed $b = 0.05$, dashed for variable b).

Based on these findings and concerning the diagrams for MAE-A, HPMC, VA64 and K30 (shown in Figure 8.3 in the appendix), it was decided to exclude the Kwei and the Couchman-Karas from the subsequent evaluation. These two models occasionally showed satisfactory agreement, yet in other systems their predictions diverged substantially from the measured values and no trend could be recognised. A similar phenomenon was observed for the Kyeremateng curves and the solubility values. In some instances, the curve exhibited a pronounced increase as it approached $x = 0$. This increase could be mitigated by adjusting the fitting parameter b instead of keeping it constant. However, this adjustment resulted in a line that was approximately parallel to the x -axis without a decline at low x -values. Consequently, the Flory-Huggins model was selected for modelling the solubility curve.

Figure 4.7 shows the plots for all six systems with only the fitted Gordon-Taylor and the Flory-Huggins curves, identified as the most suitable fits. The three storage temperatures used in stability testing, namely 8 °C, 25 °C and 40 °C, were included as orange lines. It can be seen that for all of the combinations, the typical storage conditions up to 40 °C fall within the range of phase III and IV (representing a kinetically stable glass and thermodynamically and kinetically unstable melt, respectively). Only with HPMCAS as the polymeric matrix, a small area of phase II (thermodynamically stable glass) is achievable at low drug loads.

The Gordon-Taylor curves with the fitted parameter k show a good fit for all systems except for MAE-A. The T_g -values of the drug loads 10%, 30%, and 50% deviate to a large extent from the theoretical curve with considered true densities of the components, thereby distorting the curve to a linear plot from zero to 100% drug load. Therefore, the theoretical curve was added in Figure 4.7, providing a suitable fit to high drug loads. Despite this, subsequent calculations were performed with the fitted curve.

Table 4.7 presents the limits for the single phases at the three temperatures shown in Figure 4.7, as calculated by rearranging the Gordon-Taylor equation to the drug content and by reading the intercept of the solubility curve with the respective temperatures from the plots. The lower limit shows the border between phase II and III, the upper limit between phase III and IV. For most cases, the lower limit could not be calculated, as phase II was absent at the selected temperatures.

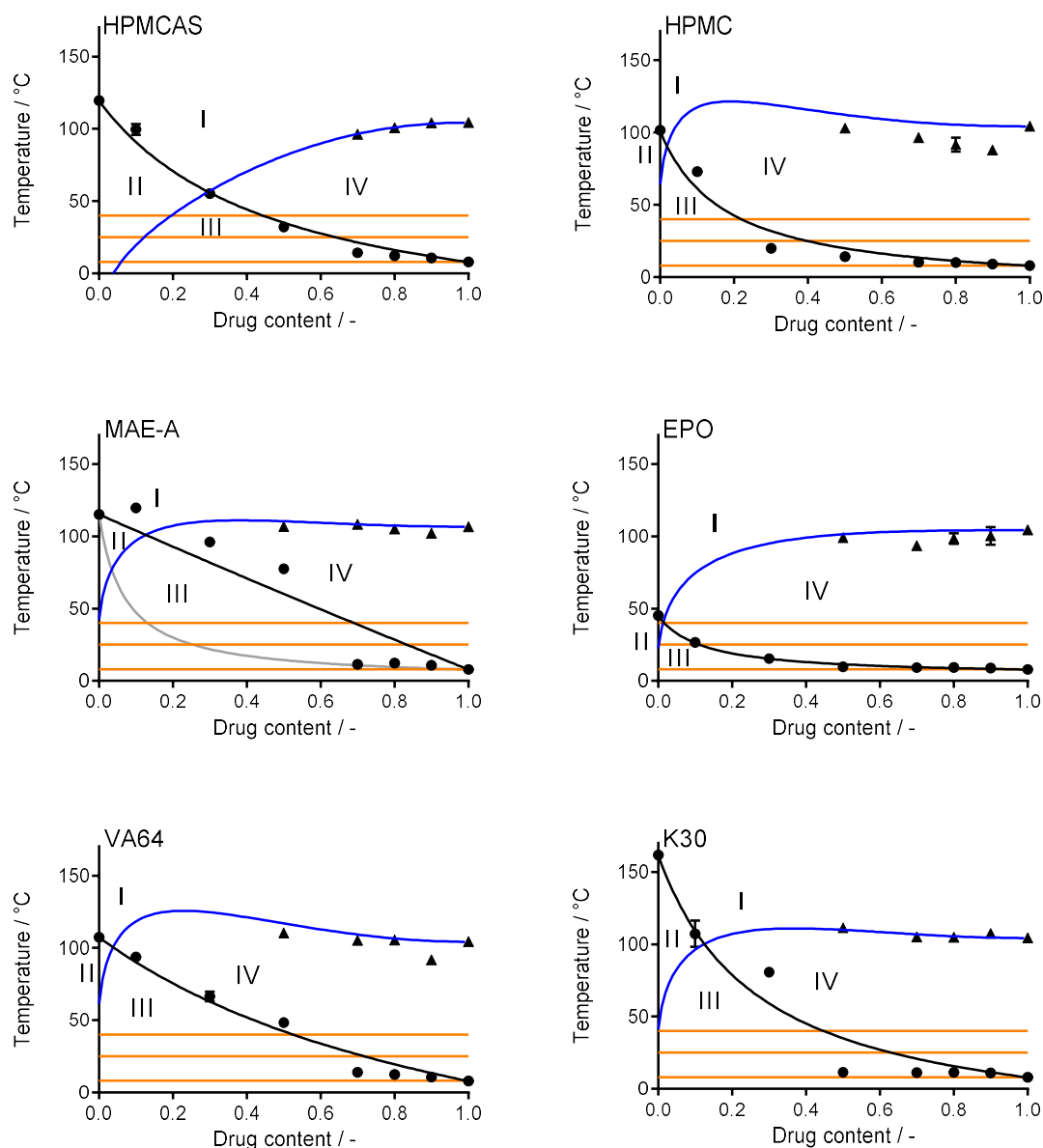


Figure 4.7 Phase diagrams of cinnarizine-HPMCAS, cinnarizine-HPMC, cinnarizine-MAE-A, cinnarizine-EPO, cinnarizine-VA64, and cinnarizine-K30 ASDs with indicated zones. Circles and triangles show the measured glass transition temperatures and solubility temperatures, respectively (mean $\pm$ standard deviation, $n = 3$). The lines represent the calculated curves for Gordon-Taylor (black; fitted k), Flory-Huggins (dark blue) and different storage temperatures (orange; 8 °C, 25 °C and 40 °C). For MAE-A, the theoretical Gordon-Taylor curve is added in grey.

Please note that in this work, the concept of a “lower limit” of solubility is applied to compare different polymeric carriers with respect to their ability to stabilise the API in the amorphous state. The lower limit is obtained by extrapolating the equilibrium solubility line into the glassy region of the binary phase diagram. While this extrapolated boundary is non-existent in practice, as the system vitrifies and falls out of equilibrium below the glass transition temperature [70], it remains a useful metric for comparative purposes. Specifically, it provides a reference against which different polymers can be evaluated in terms of their stabilising capacity. It should therefore be understood

that the lower limit does not represent an experimentally accessible stability threshold, but rather a relative indicator to visualise and compare the performance of different carriers.

Table 4.7 Drug load limits for phase III in the phase diagrams of amorphous solid dispersions with cinnarizine and MAE-E, EPO, HPMCAS, HPMC, VA64 and K30. Grey fields indicate that no lower limit could be determined due to the lack of phase II at the specific temperature.

	8 °C		25 °C		40 °C	
	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
HPMCAS	6%	100%	13%	64%	20%	44%
HPMC		99%		40%		22%
MAE-A		100%		83%		69%
EPO		96%	0%	11%	1%	2%
VA64		100%		72%		53%
K30		99%		63%		45%

At storage temperatures of 8 °C or lower, the glass transition temperature of cinnarizine has not been reached. Consequently, due to the reduced molecular mobility in the glass state, formulations with any drug load can be kinetically stabilised. For storage temperatures of 25 °C and 40 °C, a ranking for the polymers can be established, indicating the maximum drug load that can be incorporated while remaining in a kinetically stable state. At a storage temperature of 25 °C, the ranking is as follows: MAE-A > VA64 > HPMCAS > K30 > HPMC > EPO. At 40 °C, the ranking shows a slight adjustment in the order, with K30 now preceding HPMCAS. Nevertheless, at both temperatures the differences between HPMCAS and K30 are insignificant, so they can be seen as effectively equivalent: MAE-A > VA64 > HPMCAS = K30 > HPMC > EPO. It is crucial to emphasise that these calculations only apply for perfectly homogeneous systems and without consideration of any residual solvent content or water vapour adsorption.

To establish a correlation between these findings and the stability testing results of the milled extrudates, it is essential to consider that the actual cinnarizine contents in the extrudates did not always match the target values of 10%, 30%, and 50% (w/w).

It was observed that for MAE-A, all samples demonstrated stability over two years regarding crystallisation. In the phase diagrams, the maximum tested drug load of 50% can be kinetically stabilised at all storage conditions. In case of EPO, a drug load of 50% (w/w) was unstable directly after manufacturing and in the 30% (w/w) samples, crystallinity was detected at a storage temperature of 25 °C after three months. According to Table 4.7, the upper limit for drug load to achieve stabilization at 25 °C was 11 %, which corresponded with the observed results. It was not possible to analyse the EPO samples stored at 40 °C / 75% relative humidity due to melting occurring in the storage vials.

With VA64, a 50% (w/w) drug load could not form a completely amorphous system in hot melt extrusion. According to Table 4.7, it should be possible to stabilise this drug load even at 40 °C. As mentioned previously, the prerequisite for this is a perfectly homogeneous system of only two

components without water as additional plasticiser. Due to the evaporation of residual water in the process, it was assumed that there was no water in the system immediately after production. Nevertheless, the product could have subsequently ad- or absorbed water from the environment. In particular VA64 is prone to do so [57,155]. It is also possible that the extrusion did not result in a perfectly homogeneous system due to insufficient mixing, resulting in domains with higher cinnarizine content. The same seems to be applicable for K30 and HPMC samples, where a 50% (w/w) drug load could not be stabilised.

For VA64 and K30, 30% (w/w) ASDs should be stable under all tested storage conditions. This was indeed the case for K30; however, for VA64, minimal signals for crystallinity ($\leq 0.5\%$) were detected after twelve months. Since the stabilisation was only of kinetic nature, it was inevitable that a certain degree of crystallinity manifests in all systems over time. For HPMC, 30% (w/w) samples were crystalline when stored at 40 °C, as expected with the calculated upper limit being a 22% (w/w) drug load. Regarding HPMCAS, the calculated upper limits for phase III at 25 °C and 40 °C were 64% (w/w) and 44% (w/w) drug load, respectively. Samples with 30% (w/w) drug load should be stable at both conditions, consistent with findings from up to two years of stability testing. At 40 °C, only drug loads below 44% (w/w) can be kinetically stabilised according to Table 4.7. Considering that the actual drug load of the highest-loaded HPMCAS sample (where crystallinity was observed after two years at 40 °C) was $42.9 \pm 2.9\%$, the results are consistent. Concerning phase separation, the calculated phase diagrams do not distinguish between homogeneous and heterogeneous amorphous systems but assume perfectly mixed homogeneous systems. Phase separation is more likely in the thermodynamically instable regions, but cannot be predicted with certainty and can lead to more crystallisation due to enhanced nucleation and crystal growth by partial or spatial supersaturation within the solid system.

An alternative method for ranking the polymers is based on their ability to form molecular interactions with the active ingredient. Accordingly, the deviations between the measured glass transition temperature, as represented by the fitted Gordon-Taylor curve, and the theoretical curve with the true densities can be utilised. Values of Δk and ΔAUC greater than zero imply interactions between drug and polymer, while values below zero suggest that interactions within the single components predominate [30,62,65]. Table 4.8 summarises the differences of the parameter k and the area under the curve for both models (fitted minus theoretical). The Flory-Huggins interaction parameter is also included in the table. Here, negative values show the presence of intermolecular interactions. Positive values indicate that intramolecular forces are stronger than intermolecular ones.

Table 4.8 Δk and ΔAUC for Gordon-Taylor (differences between theoretical and fitted equation) as well as χ (Flory-Huggins interaction parameter) for cinnarizine amorphous solid dispersions.

		HPMCAS	HPMC	MAE-A	EPO	VA64	K30
Gordon-Taylor	Δk	0.26	0.09	0.89	-0.06	0.45	0.16
	ΔAUC	21.61	8.26	38.44	-1.61	47.18	20.79
Flory-Huggins	χ	-2.75	2.04	1.24	0.38	2.24	1.43

For Δk and ΔAUC , only the system with EPO led to negative values, indicating that interactions within the single components exert a greater influence than interactions between the API and the polymer. For the other polymers, intermolecular interactions were detected at varying strengths. For Δk , the ranking found was MAE-A > VA64 > HPMCAS > K30 > HPMC > EPO (for a comparison with rankings established by methods described above or those to follow, please refer to Table 4.16). A similar outcome was observed regarding ΔAUC , except for a shift between MAE-A and VA64. It was anticipated that interactions between cinnarizine and HPMC as well as K30 were of a weak nature. Consequently, larger deviations due to interactions were expected of MAE-A and HPMCAS, as stronger acid-base interactions, in addition to non-polar interactions, are involved in these cases. The outcome of the VA64 samples was not expected, as only weaker hydrophobic interactions were considered possible for this case. It is known from literature that VA64 can form strong interactions with active substances of weak basicity [74,155], but the superiority over the acidic polymers was unexpected. Additionally, the outcomes for HPMCAS and K30 were almost identical, both when regarding the temperature dependent drug load limits for phase III as well as the ΔAUC . With K30, only hydrophobic interactions can be formed with cinnarizine as opposed to HPMCAS. Still, the hydrophobic nature of cinnarizine seems to be strong enough to form substantial interactions with neutral polymers.

The Flory-Huggins interaction parameter was only negative for HPMCAS. There should be mostly API-API- and polymer-polymer-interactions for the other polymeric systems tested in these calculations, where the Flory-Huggins parameter was greater than zero. The result provided a ranking for χ of HPMCAS < EPO < MAE-A < K30 < HPMC < VA64, with HPMCAS having the strongest and VA64 the weakest intermolecular interactions. This is contradictory to the results presented in the Gordon-Taylor evaluation. One limitation of the Flory-Huggins fitting approach is the lack of experimental data for lower drug loads. As seen for Gordon-Taylor, especially drug loads up to 50% (w/w) are prone to deviate from the theoretical values. This range is not accessible for the Flory-Huggins evaluation using melting point depression, due to melting peak broadening by lower degrees of crystallinity in the system and due to a melting point plateau induced by increased viscosities at lower drug loads [157]. There is a tendency to overestimate the solubility temperatures for this area, leading to a distortion of χ and, subsequently, an inaccurate assessment of the presence and strength of interactions. When analysing solubility parameters and molecular dynamics simulations

concerning interactions between cinnarizine and the polymers (sections 4.2.11 and 4.2.13), the results are not in line with the findings from the Flory-Huggins interaction parameter, showing its limitation.

4.2.6 Solubility enhancement

The influence of different polymers on the solubility of cinnarizine in phosphate buffer pH 6.80 is summarised in Table 4.9. The asterisks indicate statistical significance of the changes, and the colour-coding shows the extent of concentration increase or decrease after reaching equilibrium solubility. Additionally, the pH of the solution after the experiment is provided, along with a colour coding for the pH-shift relative to the starting value of 6.80 without the addition of polymer or cinnarizine. For purposes of comparison, the solubility in certain aqueous and organic solvents is given in Table 4.10.

Table 4.9 Changes in cinnarizine thermodynamic solubility following polymer addition to phosphate buffer pH 6.80 (mean values of $n = 3$). Given is the factor of concentration increase and the pH measured while sampling. *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$.

Polymer	Solubility change factor		pH		Colour-code	
	1%	5%	1%	5%	Factor	pH-shift
MAE-A	65 (*)	4290 (**)	6.43	5.77	1-9	+ > 0.10
EPO	130 (**)	100 (**)	6.98	7.54	10-99	+ 0.06-0.10
HPMCAS	20 (***)	47 (*)	6.70	6.39	100-999	± 0.05
HPMC	35 (**)	690 (***)	6.81	6.88	1000-9999	- 0.06-0.10
VA64	15 (**)	65 (***)	6.82	6.80		- 0.11-1.0
K30	480 (***)	170 (***)	6.82	6.78		- > 1.0
PAA	150 (***)	1300 (***)	5.49	5.76		
PSSA	1050 (***)	31100 (***)	3.72	1.68		
Shellac	-/-	830	-/-	6.66		
PVAP	-/-	670	-/-	5.39		
Soluplus	78	230	6.81	6.81		
PEG 6,000	88	180	6.86	6.91		

Table 4.10 Saturation solubility (thermodynamic) of cinnarizine in various organic and aqueous solvents (mean ± standard deviation, $n = 3$). For organic solvents, the solubility is given in mg/mL; for aqueous solvents, the values are given in µg/mL.

Solvent	Solubility [mg/mL]	Solvent	Solubility [µg/mL]
Methanol	9.1 ± 0.9	Water	0.43 ± 0.33
Ethanol	9.5 ± 2.4	Phosphate buffer pH 6.8	0.25 ± 0.00
Octanol	17.7 ± 2.1	FaSSIF pH 6.8	5.5 ± 0.45
DMSO	0.5 ± 0.0	1 mM HCl	210 ± 5.8
Acetonitrile	11.7 ± 0.3	10 mM HCl	810 ± 27
Tert-butyl alcohol	16.5 ± 0.3	0.1 M HCl	1470 ± 50

It can be seen in Table 4.9 that all tested polymers led to an increase in the solubility of cinnarizine. The effect was concentration dependent for all polymers except for EPO and K30. For EPO, the effect can be attributed to the alkaline nature of the polymer in contrast to neutral or acidic ones. The EPO did not dissolve completely in phosphate buffer at a concentration of 50 mg/mL, so the actual concentration was not known. For the polymers with a concentration dependent solubility

improvement, the results of the 50 mg/mL polymer experiments could not be predicted from the values obtained for the 10 mg/mL experiments. The factor for solubility enhancement did not increase by a factor of five, but sometimes less (e.g., HPMCAS) and sometimes significantly more (e.g., MAE-A and PSSA).

PEG and Soluplus were additionally tested in regards to their ability to increase cinnarizine's solubility. Soluplus serves as solubiliser and PEG is a co-solvent, both without acidic or basic functional groups (see Figure 8.4 in the appendix). Both did enhance the thermodynamic solubility of cinnarizine to a higher degree than HPMCAS, HPMC, and VA64. Solubilisers do not interact with the solute, but form micelles that can disperse in the aqueous medium with the lipophilic solute entrapped. The polymers shellac and PVAP were only tested on a 5% level but were effective in increasing the solubility. Both polymers possess acidic structures, shellac in form of aleuritic acid, shelloic acid and jalaric acid, and PVAP in the phthalate moiety.

Because of the strong pH-dependence of cinnarizine's solubility, the interpretation of polymer-induced solubility enhancement requires consideration of potential pH effects. In this analysis, the solubility change factor was calculated relative to the solubility at pH 6.8, and the corresponding pH shifts were reported. Figure 4.8 shows the solubility data points together with a pH-dependent cinnarizine solubility. Only solutions with a cinnarizine concentration significantly higher than this line showed an actual solubility increasing effect due to the polymer. Nonetheless, since the acidic or alkaline properties of polymers directly influence the dissolution environment *in vitro* and *in vivo*, their contribution cannot be disregarded. In this sense, polymers may enhance solubility both by direct molecular interactions with the drug and by indirectly modulating the local pH conditions.

At a concentration level of 5% polymer addition, all polymers increased the solubility of cinnarizine compared to acidified water with an identical pH, except for HPMCAS. Here, an increase compared to the pure API without a shift in the pH is given, but this effect seems to be only pH-related. In addition to the pH-shift, the other polymers possess an additional effect on solubility. This is also the case for 1% polymer concentration of K30, EPO, PEG, HPMC, and VA64, while for PSSA, PAA, MAE-A and HPMCAS, the effect seems to be mainly pH-based. Once more, the solubility is elevated in comparison to phosphate buffer without polymer addition, but by no more than the degree induced by a reduced pH.

For the basic polymer EPO, the pH is increased upon dissolution. This is also the case for PEG, which itself does not affect the pH, but by dissolving the weakly basic cinnarizine, the pH rises.

Regarding the six main polymers, considering the 10 mg/mL addition, the influence on the solubility of cinnarizine was ranked as follows: K30 > EPO > MAE-A > HPMC > HPMCAS > VA64. For 50 mg/mL polymer concentration, the ranking was: MAE-A > HPMC > K30 > EPO > VA64 > HPMCAS.

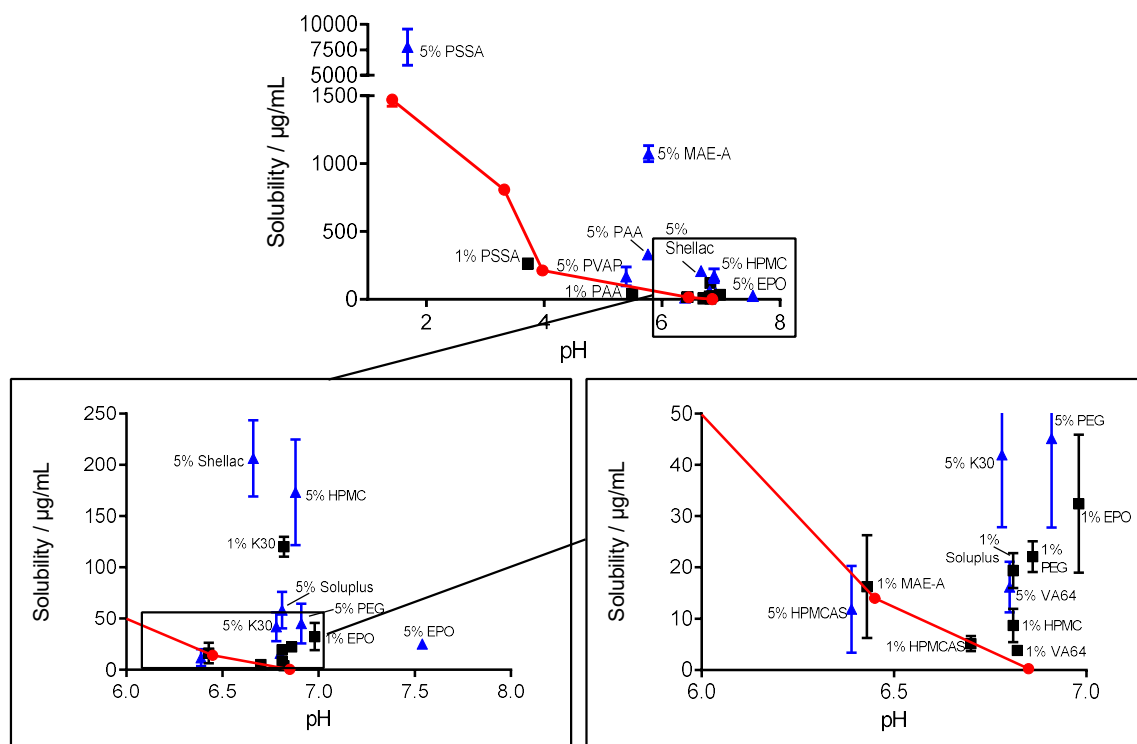


Figure 4.8 pH-dependent thermodynamic solubility of cinnarizine in phosphate buffer pH 6.8 with different polymers. The red line shows the solubility at different pH values without polymer addition as determined at different concentrations of hydrochloric acid. Black squares and blue triangles show the results for 1% (10 mg/mL) and 5% (50 mg/mL) polymer addition, respectively (mean $\pm$ standard deviation, $n = 3$). Shown are two zooms to better visualise the single data points; note that the y-axis in the upper plot is separated into two segments.

4.2.7 Partition coefficient

Although the polymers had varying effects in solubility testing, the selected ones all yielded comparable partition coefficients as shown in Figure 4.9. A reduction of the coefficient compared to that of phosphate buffer pH 6.8 as the aqueous phase was observed for all the systems; however, none of them reached values as low as 0.1 M hydrochloric acid. After polymer addition (10 mg/mL), no substantial shifts in the pH values were observed. While the experiments with hydrochloric acid showed that the partition behaviour was affected by the pH of the aqueous phase, this could not be the reason for the reduced value when adding polymers. The decrease was significant in all cases, but when comparing the values between the individual polymers, no differences were found; thus, no ranking of the polymers could be determined.

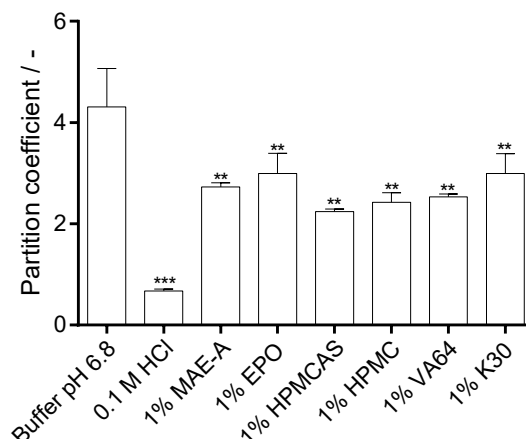


Figure 4.9 Partition coefficient of cinnarizine depending on the polymer addition to the aqueous phase. The organic phase was octanol in all cases; when polymers were added to the buffer in the aqueous phase, the concentration was 1% (w/w). The displayed values represent the mean $\pm$ standard deviation ($n = 3$). *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$, all referring to the comparison with phosphate buffer without polymer addition.

4.2.8 Dissolution testing – USP Apparatus II

4.2.8.1 Crystalline cinnarizine

For the purposes of comparison, the crystalline substance was subjected to analysis at two distinct levels of supersaturation. The addition of 20 mg and 100 mg cinnarizine to the dissolution vessel was equivalent to a supersaturation by a factor of 20 or 100 relative to the saturation solubility of approximately 5 $\mu\text{g/mL}$. Comparable dissolution curves fluctuating around the solubility limit resulted (Figure 4.10). The deviations were significantly larger adding 100 mg as seen with 20 mg, which was attributed to the potential sampling of crystalline particles, which dissolved in the subsequent centrifugation step. The crystalline substance alone did not result in a supersaturation of the solution.

The melt-extruded cinnarizine, with a residual crystallinity of 26.7%, performed poorly in dissolution testing. The increase in concentration was significantly slower than for the unprocessed crystalline substance. Even after four hours, the thermodynamic saturation solubility was not reached (see Figure 4.10). However, the extruded product had to be given to the dissolution vessel as a single piece, as milling was not feasible due to the low T_g of only 8 $^{\circ}\text{C}$. The difference in dissolution performance could be attributed to the differences in particle size and specific surface area. Additionally, a white colouring on the surface of the extruded product after dissolution testing implies that crystallisation may have occurred here, hence forming a dissolution barrier. Given the reduced specific surface area of the extruded product compared to the powdered samples, a more precise comparison could be made with the VCM discs instead.

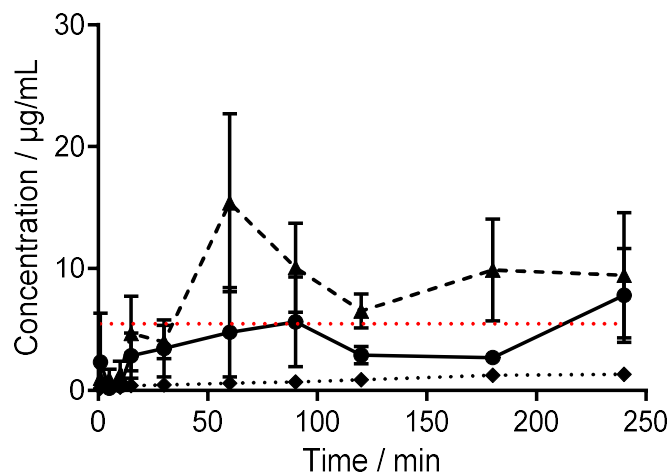


Figure 4.10 Dissolution profiles of cinnarizine (20 mg, circles with solid line and 100 mg, triangles with dashed line) and extruded cinnarizine (rhombuses with dotted line). Displayed are the mean $\pm$ standard deviation ($n = 3$); the red dotted line shows the thermodynamic solubility.

4.2.8.2 Influence of shape

The impact of the specific surface areas on the dissolution performance was also evident when comparing the VCM discs with the milled extrudates of identical composition. The discs showed a substantially lower amount of dissolved drug, reflected in a smaller AUC in a four-hour dissolution experiment. Figure 4.11-A shows examples of 10% (w/w) drug load in HPMCAS, HPMC and MAE-A for both discs and milled extrudates. At this drug load, cinnarizine was completely amorphous in all tested systems. While the discs did not reach the solubility limit, the milled extrudates demonstrated drug release beyond the saturation solubility. Furthermore, supersaturation was sustained until the end of the testing after four hours.

Figure 4.11-B depicts the curves obtained with EPO-, K30- and VA64-ASDs. EPO-ASDs did not release the drug in quantities above the saturation solubility, both from discs and extrudates. For povidone K30, the AUC was nearly the same for the 10% (w/w) drug load of the discs and the extrudates. For VA64, the AUC was substantially higher for the discs compared to the milled extrudates with larger specific surface area. During the initial fifteen minutes, the curves were congruent. Then, the milled extrudates showed a steeper decline down to the solubility limit. The milling process did not only lead to a larger specific surface area for dissolution, but also to an increased surface area more prone to recrystallisation. This phenomenon was observed exclusively for VA64. In literature, it is described that crystal seeds significantly decrease dissolution and enhance further crystallisation on the formulation's surface, and that crystallinity on the material's surface is more crucial than overall crystallinity regarding the dissolution rate [84,158]. The API concentration in the dissolution experiments with VA64 milled extrudates and K30 discs were equal to the thermodynamic solubility of cinnarizine after four hours of testing. This shows that the setup

and duration of the experiments was appropriate to determine the two phases of spring and parachute, where applicable.

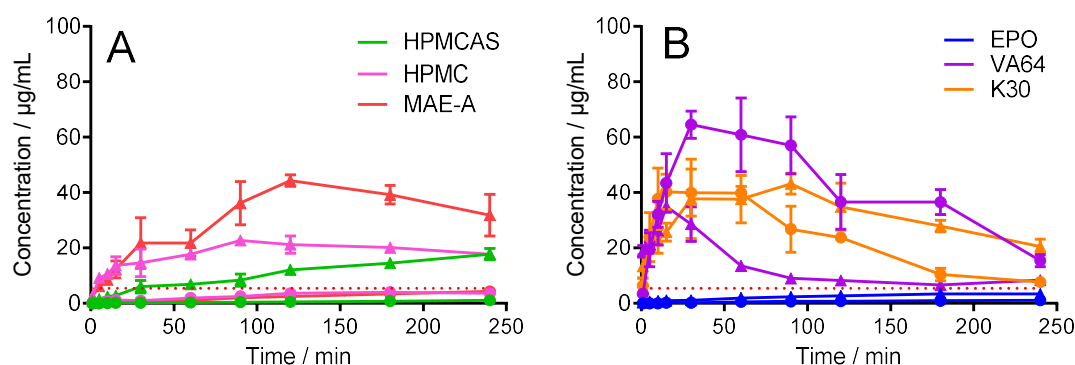


Figure 4.11 Dissolution profiles of milled cinnarizine extrudates (triangles) and VCM discs (circles) in FaSSIF pH 6.8. Shown are HPMCAS (green), HPMC (pink), MAE-A (red), EPO (blue), VA64 (purple), and K30 (orange) amorphous solid dispersions, each with 10% (w/w) drug load. Displayed are the mean $\pm$ standard deviation ($n = 3$). The red dotted line shows the thermodynamic solubility of cinnarizine. A release of 100% was equivalent to 100 $\mu\text{g/mL}$.

Figure 4.12 summarises the AUCs for the tested ASDs at 10% and 50% (w/w) drug load. As seen in the examples before (Figure 4.11), only for VA64 the AUC was larger for the VCM discs compared to the milled extrudates (with $n = 3$, where each data set originates from three independent discs or three independent weighings of ground extrudates, respectively). Although the dissolution rate was initially identical, disparate recrystallisation behaviour resulted in a different curve shape and therefore a smaller AUC for the milled extrudates.

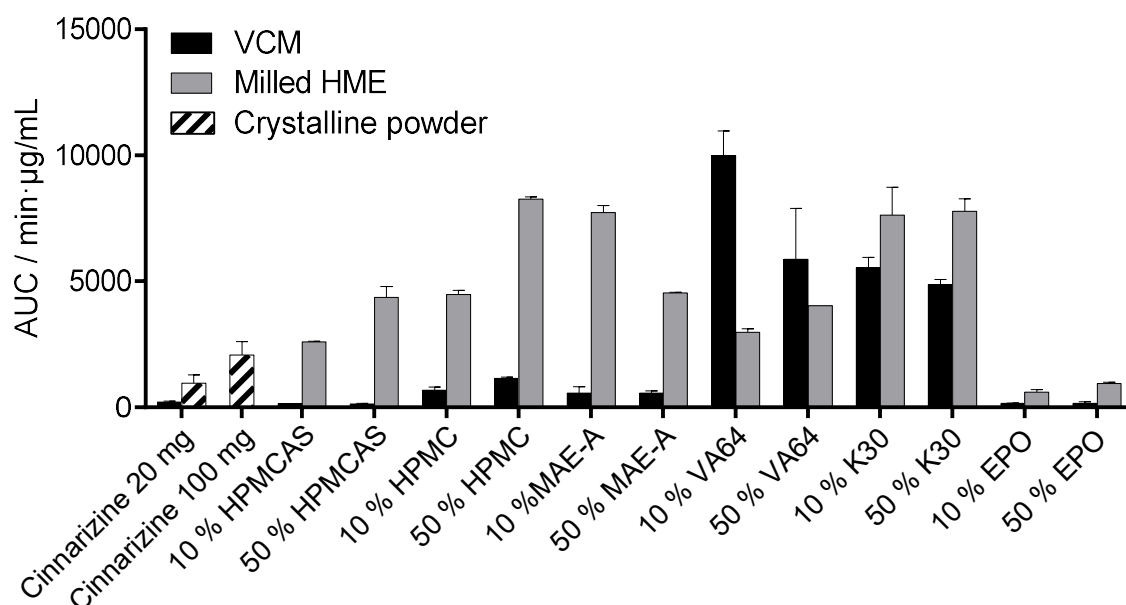


Figure 4.12 AUC of dissolution profiles of cinnarizine ASDs from $t = 0$ min to 240 min. The results are shown for VCM discs (black columns), milled extrudates (grey columns) and crystalline powder (hatched columns). Displayed are the mean $\pm$ standard deviation ($n = 3$).

4.2.8.3 Influence of drug load

It can be seen in Figure 4.12, that the drug load did not influence the release behaviour for the poorly performing polymers processed to VCM discs, being EPO, MAE-A, HPMC, HPMCAS. Drug release was found to depend on polymer dissolution, rather than the amount of drug remaining in the reservoir. For VA64 and K30, the AUC was larger for 10% (w/w) drug load compared to 50% (w/w) in the discs. Figure 4.13-A shows the results for VA64. Both the 50% (w/w) discs and the milled extrudates exhibited a consistent drug concentration, with a constant supersaturation by a factor of approximately two for the extrudates and approximately four for the discs. In contrast to the 10% (w/w) samples, the 50% (w/w) samples were not completely amorphous with a residual crystallinity of approximately 12%. Therefore, a large supersaturation might be missing here. Although recrystallisation is occurring here as well, the drug concentration remained relatively constant due to the larger reservoir of undissolved drug available. The 10% (w/w) samples reached supersaturation to a larger extent for the VCM discs than for the milled extrudates. The course of the curves was discussed above, explaining the differences based on the faster recrystallisation for the larger specific surface area.

For K30, the behaviour of the discs was the same as for VA64 (see Figure 4.13-B). Supersaturation was slightly larger for the 10% (w/w) samples compared to the 50% (w/w) ones, resulting in a larger AUC. The residual crystallinity in the 50% (w/w) samples with K30 was 8%. For VA64 and K30, different effects were observed with the milled extrudates. Here, the AUC was equal for 10% and 50% (w/w) samples with K30, while the 50% samples with VA64 resulted in slightly more drug being dissolved. In the K30 samples, the 50% (w/w) milled extrudates led to a larger level of supersaturation by a factor of > 20 , despite residual crystallinity, but the areas were equal after four hours of testing due to a more rapid decline. Nonetheless, it was expected that the 50% samples would yield significantly larger AUCs due to the amount of drug that is available for dissolution.

The milled extrudates of HPMCAS, HPMC, and EPO showed an increase in drug dissolution with an increase in drug load, reflected by a higher AUC. Even though the total amount of drug release was higher for the samples with 50% (w/w) drug load in comparison to the ones with 10% (w/w), the full potential of the higher drug load could not be achieved, as the proportion of the released drug in relation to the total amount was lower for the 50% (w/w) samples.

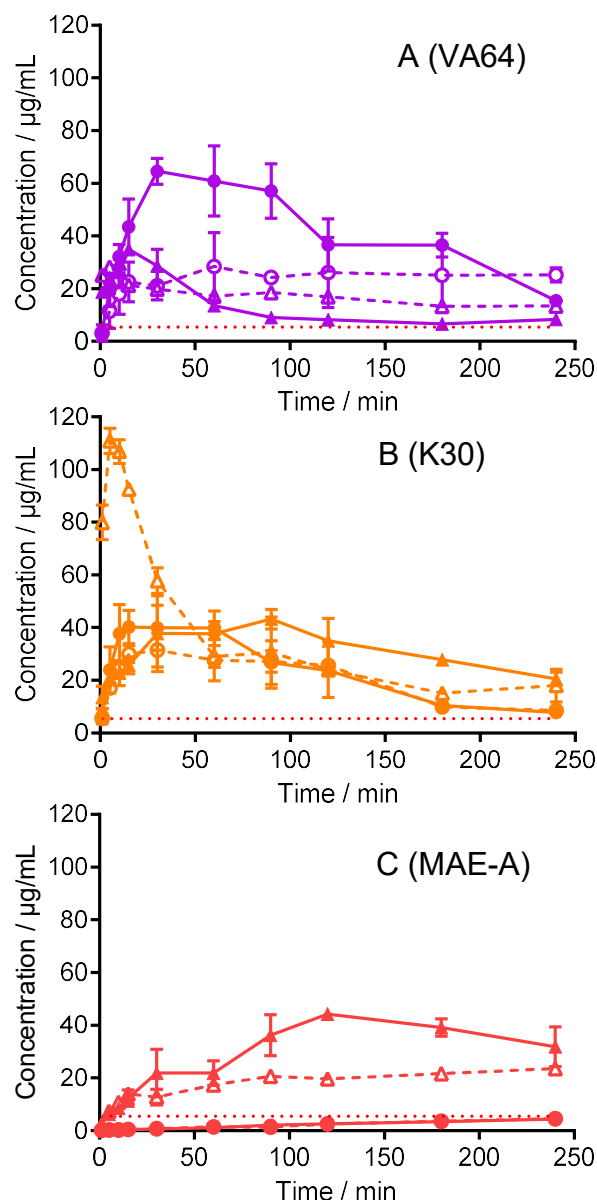


Figure 4.13 Dissolution profiles of milled cinnarizine extrudates (triangles) and VCM discs (circles) in FaSSIF pH 6.8 for VA64 (A), K30 (B) and MAE-A (C). Shown are 10% (w/w) drug load (filled, solid line) and 50% (w/w) drug load (empty, dashed line). Displayed are the mean $\pm$ standard deviation ($n = 3$). The red dotted line shows the thermodynamic solubility of cinnarizine. For MAE-A, the 10% and 50% VCM disc curves are congruent. A release of 100% was equivalent to 100 $\mu\text{g/mL}$ for 10% samples and to 500 $\mu\text{g/mL}$ for 50% samples.

For MAE-A, the AUC was substantially higher for the 10% (w/w) milled extrudates compared to the 50% (w/w) ones. The 50% (w/w) samples were fully amorphous. Figure 4.13-C shows the dissolution profiles. Both systems release cinnarizine to the same extent for the first 90 minutes. Afterwards, the concentration for the 50% (w/w) samples stayed almost constant, whilst the release for the 10% (w/w) samples continues to increase until 120 minutes, followed by a decrease due to recrystallisation. The higher amount of polymer in the samples with lower drug load may have promoted more interactions between the drug and the polymer that are favourable. This could be

beneficial for stabilising the supersaturated state. Additionally, it is possible that cinnarizine crystallised in the 50% samples within the solid particles during the dissolution process, once the polymer had started to dissolve. In the system with a 10% drug load, however, more polymer is available to stabilise the amorphous active ingredient when part of the polymer has dissolved, as the dissolution process is congruent [36,159].

In the samples with 50% (w/w) drug load, the greater availability of drug molecules increased the likelihood of forming clusters of higher concentration, which are prone to recrystallisation. However, a sharp decrease in concentration due to recrystallisation is missing in the 50% samples. This could be explained by the alternating effect of higher drug load leading to more crystallisation, while simultaneously providing a greater reservoir for further dissolution.

4.2.8.4 Influence of polymer functional group

In addition to the drug load and the geometrical shape of the formulation, the impact of different polymer types was investigated in this study. Figure 4.14 presents a classification of the tested polymers based on their functional groups. Due to the weakly basic nature of cinnarizine, it was hypothesised that acidic polymers would have the most beneficial impact on its dissolution performance. The ionic interactions between these components were presumed not only to stabilise the amorphous state in solid form, resulting in an increased release rate, but also to maintain a supersaturated state in solution by inhibiting precipitation. In contrast to the initial expectations, the neutral polymers had the strongest effect on the extent of release and stabilisation of the supersaturated state of cinnarizine and therefore led to the highest AUCs. It seems unlikely that the effect was based on an increased saturation solubility in the medium alone. Even if the polymers were to be fully dissolved, the resulting concentrations would be only 0.05% and 0.09% for the 50% and 10% samples, respectively. Studies on solubility improvement showed only minor effects on a 1% concentration level for the tested polymers compared to phosphate buffer (see chapter 4.2.6). The initial solubility in FaSSIF was already higher. Furthermore, the polymers did not dissolve completely at the beginning of the testing but rather dissolved at varying speeds. This effect was even more apparent for the VCM discs: only the discs made of K30 and VA64 dissolved completely, while the others remained largely intact after dissolution testing. Figure 4.15 supports this observation. The dissolution speed of the polymers was tested in phosphate buffer pH 6.8 via turbidity analysis. It was found that solely K30 and VA64 achieved complete dissolution over the four-hour testing period, while MAE-A and HPMC left residual particles in the solution. For HPMCAS and EPO, the remaining turbidity was clearly visible, though HPMCAS is completely soluble at the given conditions. The polymer swells upon contact with the aqueous medium, forming a gel-like layer that further decreases dissolution speed. The rate of polymer dissolution appears to have a larger influence on the overall dissolution performance than the interactions between the components or the stabilisation of the amorphous form in the solid state. The enhanced disintegration of systems with VA64 and K30 compared to other polymers contributes substantially to their superior dissolution

performance. Still, they are also capable of stabilising a supersaturated state as shown previously in the dissolution plots. It should be noted that the polymer dissolution analysis was performed in phosphate buffer, which does not fully replicate the physiological environment of FaSSIF. Turbidity measurements in FaSSIF were not feasible due to its inherent turbidity from bile salts and lecithin. Visual observations of VCM discs in FaSSIF at 37 °C showed disintegration and dissolution patterns that qualitatively agree with the pH-dependent polymer solubility trends identified in the buffer-based assay. Therefore, while the absolute kinetics may differ in the intestinal environment, the assay provided a comparison of polymer dissolution behaviour and supported the interpretation that polymer solubility plays a key role in ASD performance.

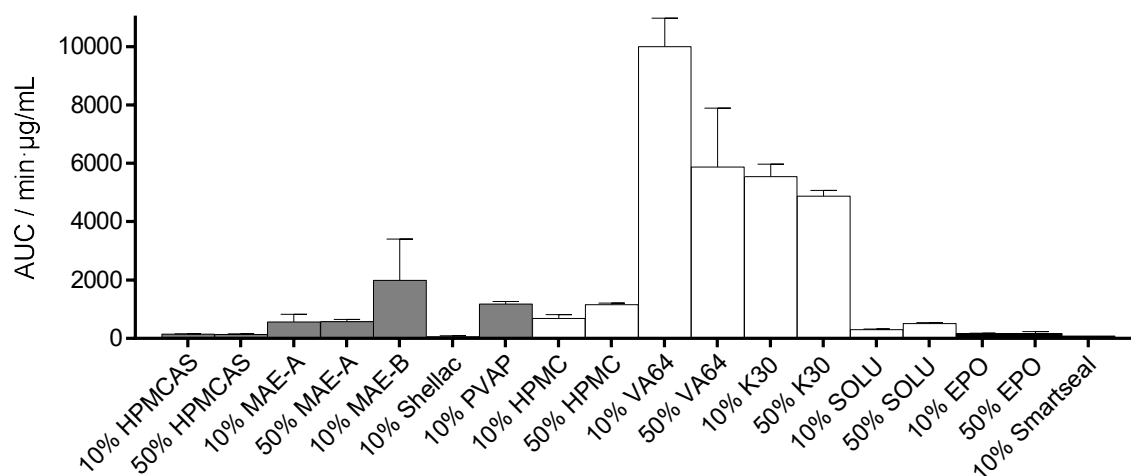


Figure 4.14 Area under the curve of the dissolution profiles of cinnarizine VCM discs from $t = 0$ min to 240 min sorted by polymer type. Shown are polymers with acidic (grey), neutral (white) and basic (black) functional groups, each mean $\pm$ standard deviation, $n = 3$.

Kaushal et al. found in a study that for carrier-controlled drug release, strong interactions between the active and the polymer could hinder drug dissolution [160]. This is the case for HPMCAS and HPMC due to the discussed gel formation upon swelling. Here, cinnarizine needs to diffuse through the formed gel layer. In this step, strong interactions with the gel-forming agent can slow down the diffusion process, which explains why the transport through the HPMCAS-matrix is slower compared to the one in HPMC. With MAE-A, the effect is not as pronounced as for HPMCAS, as the polymer dissolves instead of swelling and therefore, the distance for cinnarizine molecules to diffuse is smaller. Once dissolved from MAE-A or HPMCAS systems, the supersaturated state in solution is stabilised by the two polymers due to their stronger interactions with cinnarizine compared to VA64 or K30. For VA64 and K30, a higher level of supersaturation in the spring-region was observed, but due to less interactions and stabilisation potential, the parachute-effect was not as strong as for the acidic polymers, where no decrease in drug concentration was observed over the whole tested time.

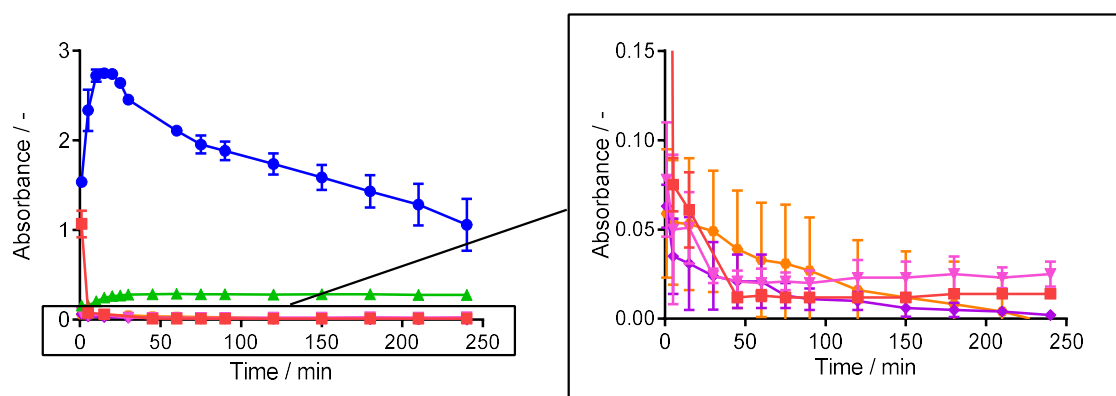


Figure 4.15 Dissolution speed of polymers as determined via turbidity analysis over 240 min in FaSSIF pH 6.8 buffer. The inserted image section shows a larger scaling on the y-axis. Shown polymers are MAE-A (red squares), EPO (blue circles), HPMCAS (green triangles), HPMC (pink inverted triangles), VA64 (purple rhombuses) and K30 (orange hexagons), each mean $\pm$ standard deviation, $n = 3$.

The AUC of cinnarizine dissolution from 10% (w/w) milled extrudates decreased within the series MAE-A > K30 > HPMC > VA64 > HPMCAS > EPO. For 50% (w/w) milled extrudates, the order was HPMC > K30 > MAE-A > HPMCAS > VA64 > EPO. For VCM discs, the ranking was VA64 > K30 > HPMC > MAE-A > EPO > HPMCAS for both drug loads. Although there were variations, there was a general trend that EPO and HPMCAS performed worst, which fits to the findings on the dissolution speed of the polymers. Here, the AUC ranking was VA64 < K30 < HPMC < MAE-A < HPMCAS < EPO, with VA64 having the fastest dissolution speed and EPO the lowest.

4.2.8.5 ASDs prepared by spray drying

The dissolution studies show substantial differences in the outcomes of formulations prepared by spray drying with VA64 and MAE-A (50% drug load each, see Figure 4.16). In the formulation of VA64, the concentration remained only slightly above the saturation solubility, lacking significant supersaturation and crystallisation or concentration changes. The concentration increased up to 30 minutes of testing and then remained constant. For MAE-A, it exhibited supersaturation characteristics of a spring and parachute profile, with the maximum supersaturation factor of approximately ~ 10 occurring after 60 minutes, followed by subsequent declines, while supersaturation lasted for up to 4 hours.

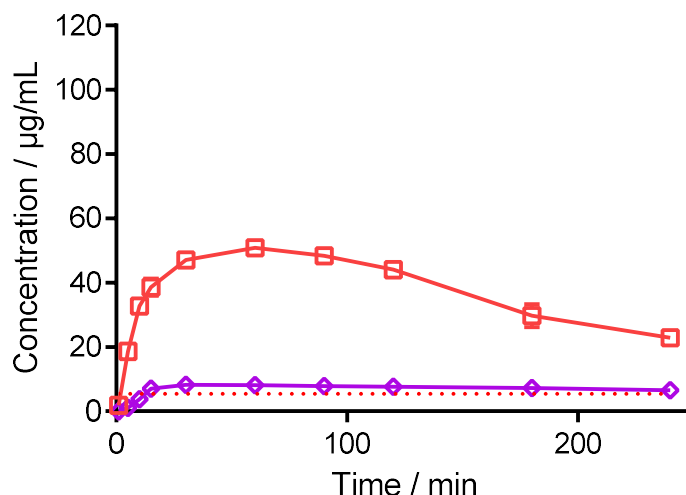


Figure 4.16 Dissolution profiles of spray-dried cinnarizine-polymer systems in FaSSIF pH 6.8 for VA64 (purple rhombuses) and MAE-A (red squares). Shown are 50% (w/w) drug load. Displayed are the mean $\pm$ standard deviation ($n = 3$). The red dotted line shows the thermodynamic solubility of cinnarizine. A release of 100% was equivalent to 500 $\mu\text{g/mL}$.

These profiles did not mirror the results of the melt-extruded systems where VA64 showed a high supersaturation and the cinnarizine concentration released from MAE-A systems only slowly increased. Comparing the AUCs, the differences become even more pronounced with 9120 $\text{min}\cdot\mu\text{g/mL}$ (SD) versus 4550 $\text{min}\cdot\mu\text{g/mL}$ (HME) for MAE-A and 1730 $\text{min}\cdot\mu\text{g/mL}$ (SD) versus 4030 $\text{min}\cdot\mu\text{g/mL}$ (HME) for VA64.

The residual crystallinities were 2.3% (SD) versus 0% (HME) for MAE-A and 14.0% (SD) versus 21.6% (HME) for VA64. Though crystallinity was higher for the melt-extruded systems, the dissolution rate was also higher. For MAE-A, crystallinity was slightly higher after spray drying, but also the drug release was higher for the spray-dried samples. The release rate did not correlate with crystallinity in this instance.

Another factor influencing drug release is the available specific surface area, which increases with a reduction in particle size. Figure 8.12 in the appendix shows the particle size distributions of the ASDs prepared by both hot melt extrusion (after milling) and spray drying. The d_{50} -values obtained were $<10\ \mu\text{m}$ for spray-dried MAE-A and VA64 ASDs with 50% drug load. The milled extrudates of VA64 with 50% drug load showed a comparable size distribution. 50%-loaded MAE-A milled extrudates had a larger median (d_{50}) of $17\ \mu\text{m}$. This could explain the faster drug release from spray-dried samples with MAE-A. It is known from literature that particle size can have a significant influence on drug release from amorphous solid dispersions, with dissolution rate being inversely proportional to particle size [161,162].

4.2.8.6 Ternary ASDs

In the hot melt extrusion formulations, the use of VA64 resulted in a high degree of supersaturation and a pronounced improvement in the release profile, while MAE-A showed a more prolonged but constant release behaviour. In contrast, ternary systems with both polymers combined from HME and spray drying exhibited uneven dissolution characteristics, with large deviations and no clearly defined release curve (Figure 4.17-A). Supersaturation levels in the ternary dispersions consistently remained below a factor of approximately four, showing an initial rapid increase followed by a sharp decline, and later a slight, fluctuating rise. Overall, these ternary systems did not provide any additional benefit compared to VA64-based binary formulations alone. The ternary system obtained in spray drying had a more stable dissolution profile with a consistent decrease from the maximum point, whilst in the HME formulation, fluctuations dominated the course. The profile of the spray-dried formulation resembles the course of the spray-dried MAE-A formulation, but with lower supersaturation and lower final concentration (compare with Figure 4.16).

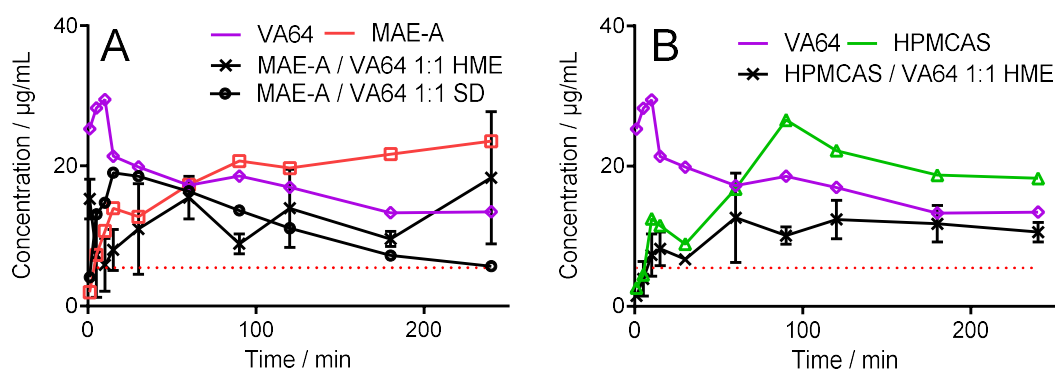


Figure 4.17 Dissolution profiles of ternary cinnarizine-polymer systems in FaSSIF pH 6.8. Shown are melt-extruded systems of MAE-A/VA64 1:1 (A) and HPMCAS/VA64 1:1 (B) and the spray-dried system of MAE-A/VA64 1:1 (A), each with 50% drug load, together with the respective binary systems from HME. Displayed are the mean $\pm$ standard deviation ($n = 3$). The red dotted line shows the thermodynamic solubility of cinnarizine. A release of 100% was equivalent to 500 $\mu\text{g/mL}$.

For the formulation with HPMCAS and VA64 prepared by HME, the ternary systems showed only a gradual increase in supersaturation, which remained limited to a factor of approximately two throughout the study. This performance was markedly lower than that of the VA64 binary dispersions, indicating that the addition of a second polymer did not provide a clear benefit in terms of supersaturation or release behaviour. VA64 alone achieved high supersaturation and demonstrated strong dissolution enhancement, whereas HPMCAS produced a slower but more constant release profile.

In all cases, the AUC of the ternary systems was lower compared to those of the respective binary ASDs. As discussed before, the solid-state stability was also not improved by using two different polymers in one system (see chapter 4.2.1). Instead of both polymers adding their positive effect on

stability and dissolution, negative aspects of both sides prevail in all examples. A review of the respective literature reveals different results regarding the benefits of ternary and binary systems. In certain cases, ternary systems are shown to be advantageous, whilst in others, binary systems remain superior [163]. A study with ketoconazole combined with HPMC and PAA showed that the physical stability and the amount of molecular interactions (determined via ^{13}C ssNMR) for ternary systems with both polymers was in between the outcomes for the respective binary systems. With HPMC, no specific interactions and poor stability were found, whereas PAA was able to stabilise amorphous ketoconazole by forming hydrogen and ionic interactions [62]. With regorafenib, binary PVP-ASDs showed a fast drug release, but poor physical stability, whilst the more stable binary HPMCAS-ASDs performed poor in dissolution testing. A combination of both polymers in a ternary ASD resulted in poor stability and dissolution characteristics. Only the external addition of HPMCAS to the dissolution medium or as additional excipient in a tablet increased the stability of binary PVP-ASDs with regorafenib [61]. Another approach in ternary ASDs is to include a surfactant instead of a second polymer, as performed in a study with aprepitant as model drug and hydroxypropyl cellulose, povidone K12 and Soluplus as polymers, each combined with either d-alpha tocopheryl polyethylene glycol 1000 succinate or poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) as surfactant. It was shown that all combinations were miscible and that the surfactants worked as plasticisers, but the crystal growth was increased by both surfactants compared to binary systems with only the polymers. Only a combination of povidone and d-alpha tocopheryl polyethylene glycol 1000 succinate results in a decreased crystal growth rate and increased dissolution kinetics [79].

4.2.8.7 Dissolution with pH shift

The dissolution performance of 10% drug-loaded milled extrudates was evaluated in a two-step pH-shift setup. Samples were first exposed to 0.01 M hydrochloric acid for 120 minutes, followed by the addition of a FaSSIF pH 6.8 concentrate to simulate intestinal conditions. Since the saturation solubility of cinnarizine in 0.01 M HCl exceeds 800 $\mu\text{g/mL}$, the acidic phase was not expected to present a solubility limitation. The formulations tested included ASDs prepared with EPO, HPMCAS, VA64, and MAE-A, alongside cinnarizine as a reference.

In the initial acidic phase, cinnarizine, EPO-based ASDs, and VA64-based ASDs all achieved complete drug release (Figure 4.18). The apparent differences in curve height during this phase were attributable to variations in the actual drug load after extrusion, as explained in section 4.2.3, rather than dissolution limitations, as shown in Figure 4.18-B. In contrast, formulations with MAE-A and HPMCAS showed incomplete drug release reaching only 17% and 9% of the nominal content, respectively. This was due to the poor solubility of these polymers under acidic conditions. As the polymers themselves did not dissolve, the drug could not be liberated effectively from the matrix.

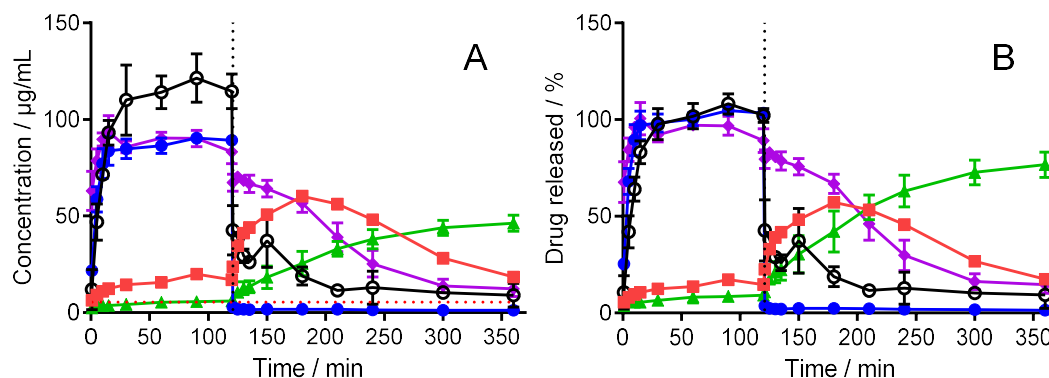


Figure 4.18 Dissolution profiles of milled cinnarizine extrudates in 0.01M HCl (120 minutes) with shift to FaSSIF pH 6.8 (additional 240 minutes; the change is indicated by the vertical dotted line). A: Drug concentration in µg/mL with the red dotted line showing the thermodynamic solubility in FaSSIF pH 6.8. B: Released drug shown as percentage. Shown are HPMCAS (green triangles), EPO (blue circles), MAE-A (red squares), and VA64 (purple rhombuses) amorphous solid dispersions, each with 10% (w/w) drug load. Displayed are the mean $\pm$ standard deviation ($n = 3$; for MAE-A, only $n = 1$ was tested). Black empty circles represent crystalline cinnarizine.

After transitioning to FaSSIF, the MAE-A formulation exhibited a pronounced spring and parachute effect. As part of the drug had already dissolved and the particles had been wetted, the initial release in the FaSSIF phase was faster compared to the experiment with only FaSSIF pH 6.8 as medium (see Figure 4.11-A for comparison). After four hours, the concentration slightly surpassed that of the crystalline reference. The HPMCAS formulation followed a different trend, showing a steady increase in drug concentration without any signs of crystallisation. Ultimately, it reached approximately 50% drug release, the highest for all formulations after four hours in FaSSIF.

The EPO-based ASDs displayed a sharp decline in concentration once the medium was switched to FaSSIF, falling below the saturation solubility and failing to recover. This behaviour was also evident in the visible precipitation within the vessel, by which crystalline API and undissolved EPO polymer formed a barrier to further dissolution. Cinnarizine drug substance also showed a sharp drop in concentration after the pH shift, though it maintained a supersaturation of about eightfold before gradually declining towards its thermodynamic solubility within four hours. The sharper decline for EPO-samples compared to the pure API can be explained by a combined precipitation of both components. The formation of polymer particles enables the enclosure of API which is no longer available as solute in the medium, and the formed particles represent a surface on which API-crystallisation is facilitated. VA64-based ASDs showed only a slight decrease after the medium switch, followed by a stable parachute effect. After four hours, the concentration was comparable to that of crystalline cinnarizine.

Table 4.11 lists the AUCs for the dissolution curves. The total release was highest for VA64, followed by cinnarizine in the crystalline state. The dissolution of the drug alone was highest in the acidic medium. The release of cinnarizine from EPO- and VA64-samples was equal in the first phase,

but the rapid concentration drop of cinnarizine with EPO after the pH-shift led to an overall lower AUC than crystalline cinnarizine and samples with VA64 and MAE-A. MAE-A and HPMCAS performed best in the second phase of drug release testing, but their negligible release in the acidic medium could not be compensated for regarding the total AUCs. As the small intestine with pH 6.8 is the main site of drug absorption, drug release here is more relevant compared to the acidic phase.

Table 4.11 AUCs for milled extrudates with 10% (w/w) cinnarizine load in pH-shift dissolution experiments with 0.01 M HCl and FaSSIF pH 6.8. All values are given in min·µg/mL as mean ± standard deviation of n = 3 (for MAE-A, only n = 1 could be analysed).

	Crystalline CIN	MAE-A	EPO	HPMCAS	VA64
0.01 M HCl	12790 ± 1230	1890	10090 ± 480	610 ± 180	10490 ± 340
FaSSIF pH 6.8	3920 ± 810	9910	370 ± 30	8160 ± 1050	8140 ± 1130
Total	16710 ± 1020	11800	10460 ± 260	8770 ± 620	18630 ± 740

4.2.9 ¹H-NMR analysis

4.2.9.1 Screening

Different ratios of cinnarizine and polymer were tested in either methanol-d₄ or toluene-d₈. Figure 4.19 shows the ¹H-NMR of cinnarizine without polymer addition in methanol-d₄. It is visible that the signals of the eight protons within the piperazine moiety merge into one broad peak with two maxima instead of clearly separated peaks. The other aliphatic protons are displayed as sharp signals with well-detectable multiplets if applicable. Aromatic protons can be found in the area between 7.0 and 7.5 ppm. The signal assignment is in accordance with a detailed analysis and summary of various NMR spectra of cinnarizine as published by Haress [164].

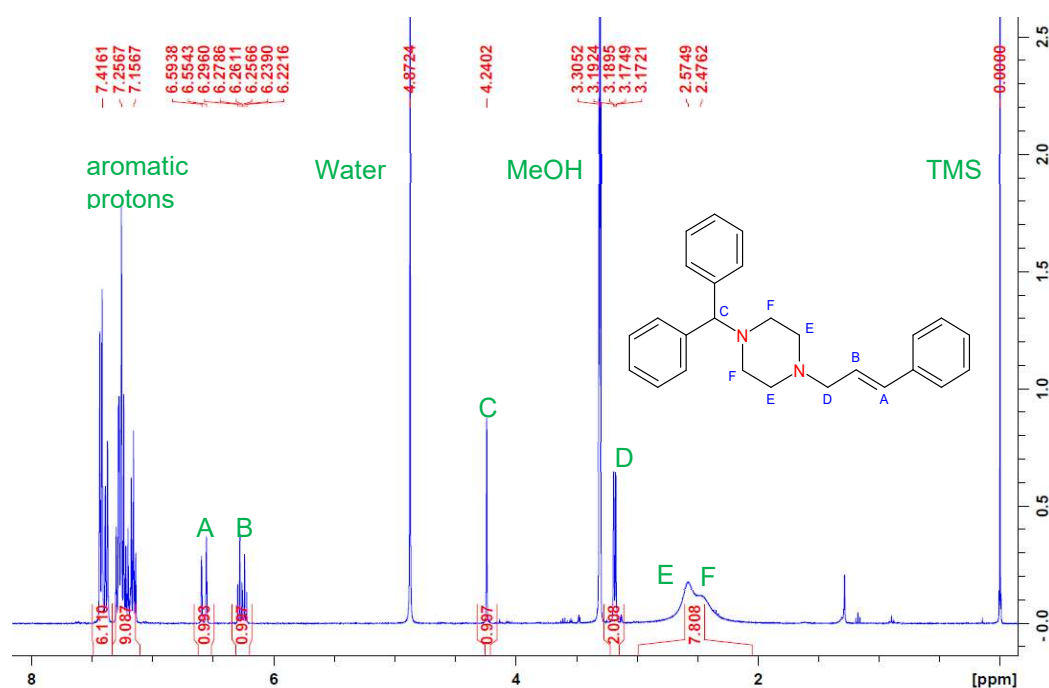


Figure 4.19 ¹H-NMR of cinnarizine in methanol-d₄ with TMS standard. Green letters show the position of protons within the molecule.

In the screening experiments with different polymers, distinct peak shift alterations were observed depending on the polymer and the solvent (Figure 4.20). In methanol-d₄, changes in the chemical shift for all proton positions except for the aromatic ones were observed when adding MAE-A or HPMCAS. The effects were of similar extents. With HPMC, only minor changes were seen. In particular, the protons in positions D and E were influenced. The changes in position D of cinnarizine together with MAE-A were of such a large extent that the peak was covered by the solvent signal and could not be evaluated. For both MAE-A and HPMCAS, the observed effects were concentration dependent. In contrast, these effects were not observed with toluene-d₈ as a solvent. When comparing the results of MAE-A in methanol-d₄ and toluene-d₈ (Figure 4.20-A and -B), the different effects are clearly visible. For HPMC, no changes were seen using toluene-d₈ either. HPMCAS could not be dissolved in toluene-d₈ in sufficient quantities.

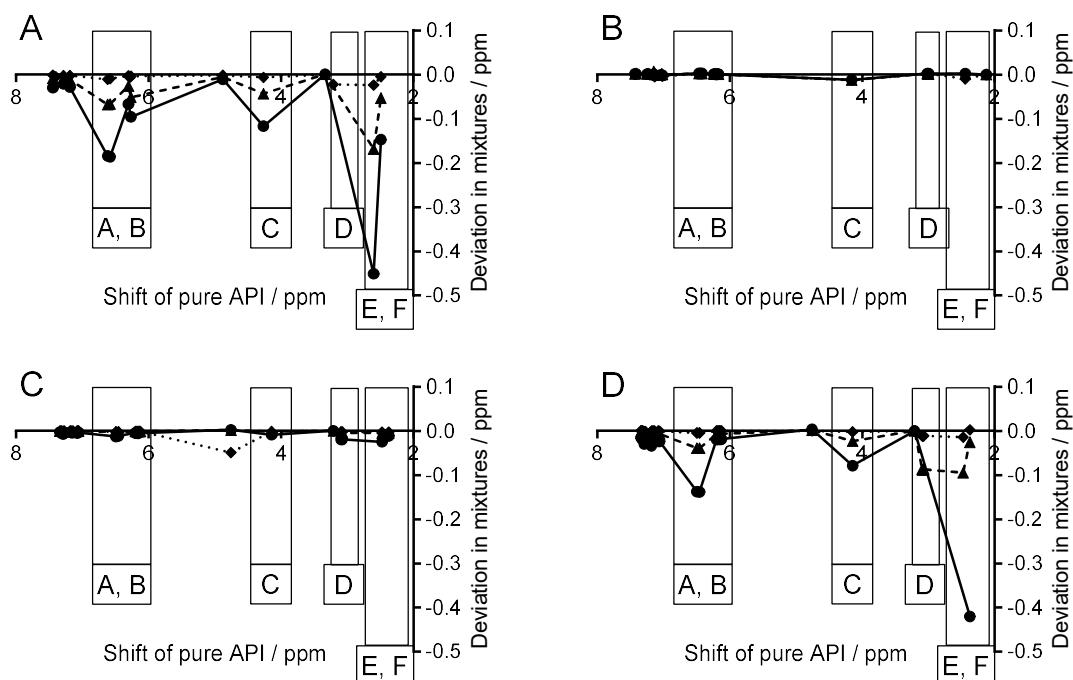


Figure 4.20 Shift of different protons of cinnarizine induced by polymer addition. Proton shift in mixtures with A: MAE-A in methanol-d₄; B: MAE-A in toluene-d₈; C: HPMC in methanol-d₄; D: HPMCAS in methanol-d₄. Mass ratios (cinnarizine + polymer) are 1+9 (solid line), 1+1 (dashed line), and 9+1 (dotted line).

The different behaviour observed in response to varying solvents can be attributed to the polar protic nature of methanol and the nonpolar aprotic characteristics of toluene. In the protic solvent methanol, proton transfers between the compounds and the solvent were facilitated. In consequence, a proton transfer from an acidic polymer such as MAE-A or HPMCAS to the weak base cinnarizine was possible. In absence of such facilitated transfer via the solvent, a direct transfer without intermediate stages would be needed which is highly unlikely. Even without a proton transfer to form a drug-polymer salt, protic solvents permit intermolecular interactions, such as hydrogen bonding. These interactions were disrupted in an aprotic solvent. This indicates that, in the context of spray

drying compared to hot melt extrusion, proton transfer processes and molecular interactions can develop more effectively in the feed solution used for spray drying. Stronger intermolecular interactions are made possible by protic solvents, like methanol, which encourage proton transfer and hydrogen bonding between the dissolved components. Conversely, because there is no liquid medium to promote proton exchange, the pre-mixed powder blend used in extrusion provides little opportunity for such interactions before melting. However, the components can become uniformly mixed and molecularly dispersed during the melting step of the extrusion process, enabling close steric contact between molecules. In this molten state, no solvent molecules, such as those found in aprotic media, interfere with molecular proximity. As a result, molecular interactions can also form within the extruded material. For spray drying, this means that the solvent selection should be based according to the desired formation of intermolecular interactions and potential proton transfers.

The deviations in the mixtures were predominantly downfield directed, meaning that peaks of the API were found at higher ppm values in the presence of polymers. In this area, the protons were more exposed to the magnetic field, given that they are less effectively shielded by the surrounding electrons. The electron density of the nitrogen atom is distributed over a larger area or more protons when it is either protonated or enters into hydrogen bonding with a polymer molecule. This results in the neighbouring protons being deshielded to a greater extent than before, leading to the discussed downfield shift.

4.2.9.2 *¹H-NMR drug polymer titration*

For the initial evaluation of the titration method with a small molecule drug as ligand to a macromolecule, bovine serum albumin was titrated with lidocaine, as binding constants are well-known for lidocaine and reported in literature (see Figure 8.5 in the appendix for the lidocaine NMR spectrum). The results for the single proton positions did not substantially vary from each other. The calculated K_D values over the whole molecule were 1.54 ± 0.42 mM (Scott), 2.65 ± 1.95 mM (Scatchard), and 1.06 ± 0.26 mM (Benesi-Hildebrand). Although all models led to comparable results, the determination with the Benesi-Hildebrand equation showed the smallest deviations. The linear fit to the datasets were best for the Scott-plot and the Benesi-Hildebrand-plot (see Figure 8.6). Literature K_D values for lidocaine and serum albumin were found to be 4.3 mM [165] and 0.1 - 1.0 mM [166].

In the further titration experiments, six different polymers were tested. An exemplary progression of the chemical shift during a titration is depicted in Figure 4.21 for MAE-A. Figure 4.22 shows the binding curves and the three applied mathematical models for the exemplary system cinnarizine - MAE-A at different proton positions (the other polymers are shown in Figure 8.7 to Figure 8.11 in the appendix). The proton positions D and E were subject to the highest shift differences, followed by A, F, C and then B. The protons on carbon number D and E are located next to the nitrogen of the piperazine moiety. Due to its basicity, the nitrogen and neighbouring atoms are

more likely to be targets for interactions with polymers, especially those carrying an acidic functional group such as MAE-A. The positions C and F are in a similar spatial relationship with regard to the second nitrogen, but the proximity to the two phenyl rings results in steric hindrance.

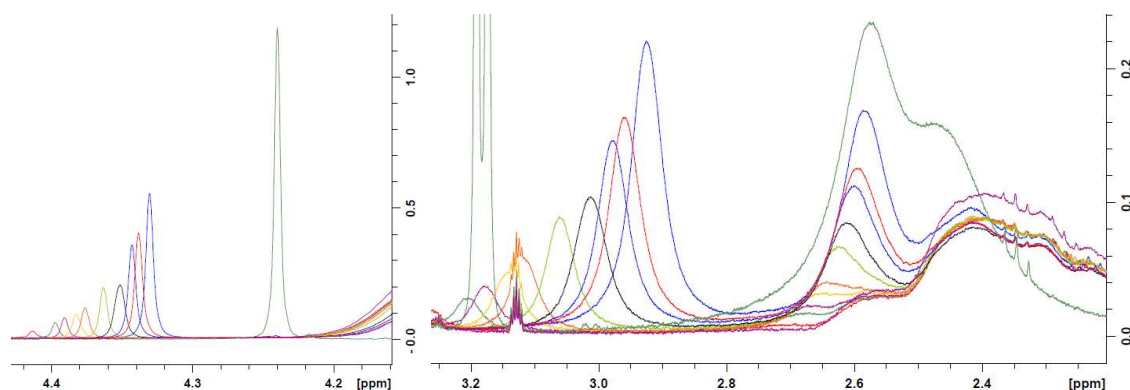


Figure 4.21 Shift of cinnarizine proton positions induced by MAE-A in methanol-d₄. The titration experiment was performed in methanol-d₄ (left: position C, right: position E and F).

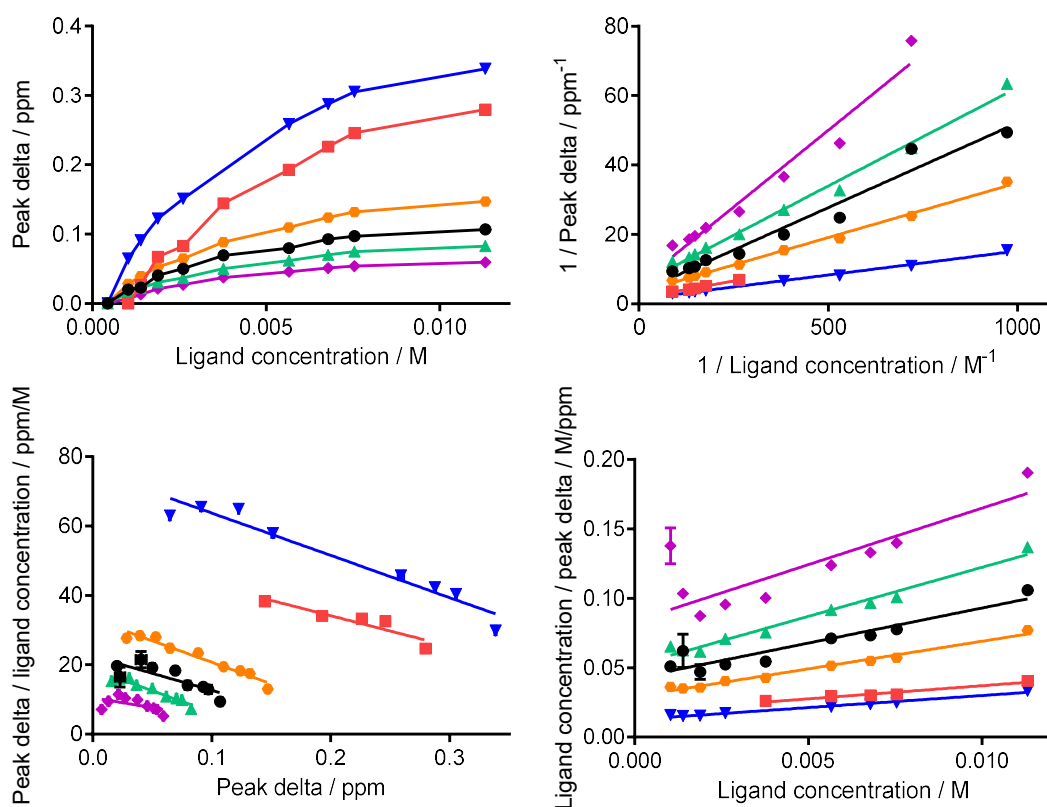


Figure 4.22 Binding curve of the cinnarizine – MAE-A titration in methanol-d₄ (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

As the presence of the polymer had the largest effect on the protons at positions D and E, these two positions were further analysed with different polymer addition. All three mathematical models were applied to compare the results. From the calculations, the parameters K_D and δ_{max} were obtained. As the K_D -value typically provides the strength of a non-covalent bond between a ligand and a macromolecule at one binding site, its evaluation was not applicable here. Polymers with equally repeating units do not possess a small number of well-defined binding sites but are prone to molecular interactions over their whole structure. If two or more very specific binding sites were present, the Scatchard plot would show two or more linear areas with different slopes. This was not observed for any of the systems. Therefore, interactions with multiple sites of the polymer were assumed. Instead of the binding constant K_D , the extent of shift changes in form of the maximum possible shift difference, δ_{max} was employed for comparison of the polymers. The calculated δ_{max} are plotted in Figure 4.23, where the maximum possible peak shift difference is shown for all six tested polymers on proton positions D and E.

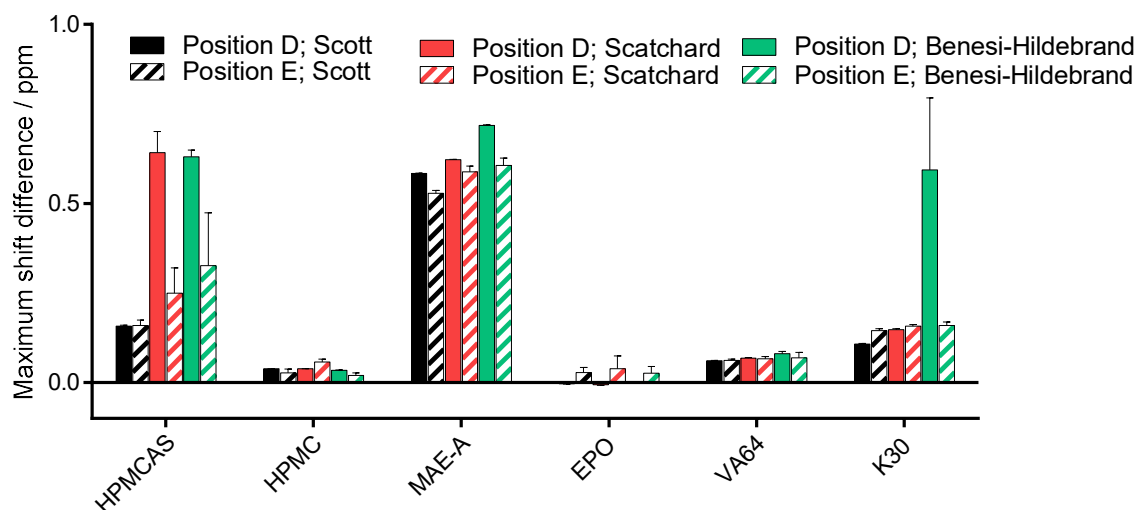


Figure 4.23 Maximum peak shift difference, δ_{max} , for proton positions D (filled columns) and E (hatched columns) of cinnarizine in dependence of the polymer. The three applied models for calculation are Scott (black), Scatchard (red), and Benesi-Hildebrand (green).

When comparing the three models and the two proton positions, the results are equal for HPMC and VA64: all models and both proton positions yield in the same results. For MAE-A, the maximum peak shift difference is slightly larger for protons on position D and the Scott-model delivers values that are marginally lower. With EPO, the shift at position D is near zero and at position E is of a negligible magnitude. With K30, the calculation for position D with the Benesi-Hildebrand-model shows a large deviation from the other outcomes for the polymer, also with a high standard deviation. The same is true for the Scatchard- and again the Benesi-Hildebrand-model for HPMCAS. The detected outliers show high standard deviations, making the results less reliable. The Scott-model demonstrated consistent performance across all polymers and proton positions.

Examining the polymers reveals a distinct contrast between systems with a high degree of interactions (MAE-A) and those with practically no interactions (EPO). To rank the polymers, it was decided to sum the δ_{max} for the positions D and E. All three models ranked the polymers MAE-A > HPMCAS > K30 > VA64 > HPMC > EPO. In reviewing individual positions, the same ranking was found, with one exception (position D, Scatchard), where δ_{max} was slightly higher for HPMCAS than for MAE-A, but also with a higher standard deviation.

Although the two polymers with acidic groups seem to be the most promising candidates for interactions with cinnarizine, an effect resulting only from the pH shift can be excluded. Differences in the chemical shift were also observed for neutral polymers, where a pH effect is not possible. While the degree of shifting might be amplified by pH influences, the same would hold true in other liquid or solid configurations where protonation or a reduced pH contribute to molecular interactions.

To check this assumption, a titration with DCl at different concentrations was performed. Figure 4.24 shows the Scott plot for positions E and D in DCl-titration. Though peak shifts were observed, they did not follow a trend and fluctuated to a large degree. To acidify the medium does not lead to a peak shift pattern according to one of the mathematical models.

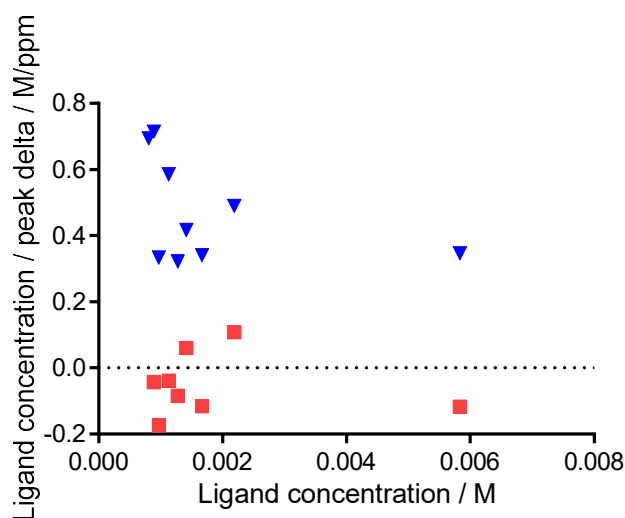


Figure 4.24 Scott plot of the cinnarizine – DCl titration in methanol-d₄. The proton positions are E (red squares) and D (blue inverted triangles).

4.2.10 Cinnarizine-HCl salt formation

The hydrochloride salts of cinnarizine were manufactured by spray drying and by vacuum drying at two different molar ratios ($\cdot$ HCl and $\cdot 2$ HCl). These methods were used to investigate the effects of the drying method and the level of protonation on the physical characteristics and solubility behaviour of the materials produced.

The successful formation of the hydrochlorides was shown by ¹H-NMR in different solvents. Data for cinnarizine hydrochloride salts showed both solvent and protonation effects on chemical

shifts, signal resolution, and differences between mono- and dihydrochloride salts (Figure 4.25). In MeOD, the peak of proton position D moved downfield and was found leftwards of the residual methanol signal opposed to the signal in cinnarizine free base. The piperazine ring proton signals exhibited more broadening and less resolution (positions E and F), but no new peaks appeared in correlation with solvent proton exchange effects.

In DMSO- d_6 , additional peaks appeared in the low-field region, including a “wave” adjacent to the low-field aromatic region, while other proton signals also moved downfield and changed in relative order. Likewise, in $CDCl_3$ there was an additional resonance in the low-field region, and the aromatic peaks were less sharp than for the cinnarizine. In both solvents, a small additional signal was found downfield right next to the aromatic signals. The additional signals can be regarded as proof for a successful protonation at two different sites for the dihydrochloride. This is supported by the lack of additional signals in methanol- d_6 , as protonation on nitrogen atoms is not visible in a protic solvent due to solvent proton exchange effects. A total yield of protonated molecules could not be determined with this method.

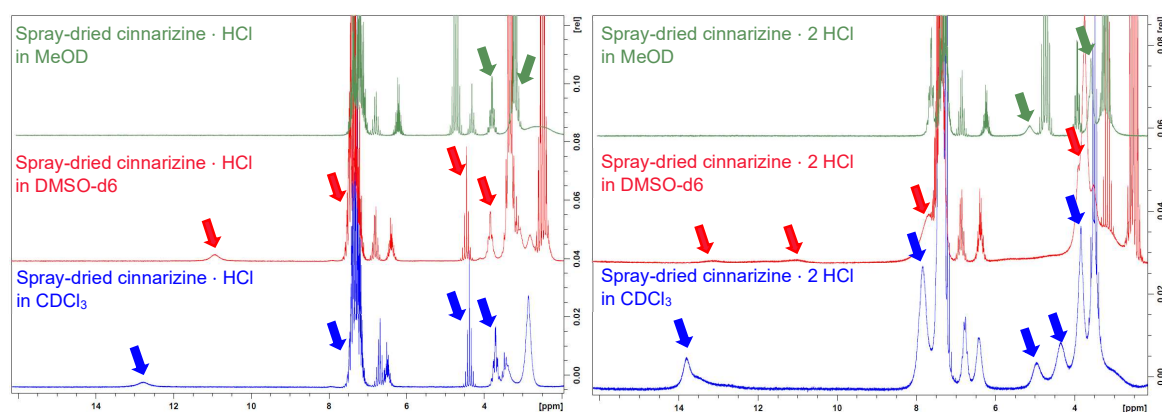


Figure 4.25 ¹H-NMR spectra of cinnarizine monohydrochloride (left) and cinnarizine dihydrochloride (right) prepared by spray drying in methanol- d_6 , DMSO- d_6 , and chloroform- d .

Substantial differences were also found between mono- and dihydrochloride salts in $CDCl_3$. The downfield signal of the dichloride at 13.81 ppm was at 12.81 ppm for the monohydrochloride and the other signals were less sharp in the dihydrochloride. In the dihydrochloride sample, there was a signal adjacent to the aromatic region (7.85 ppm) that did not appear in the monohydrochloride sample. Here, only a small wave was visible. In DMSO- d_6 , the monohydrochloride showed a single downfield resonance at 10.93 ppm, while the dihydrochloride showed two separate peaks at 13.11 ppm and 11.01 ppm, with again sharper and better resolved signals for the monohydrochloride. In MeOD, the ordering of the signals was changed for both salts, but resolution was still better on the monohydrochloride compared to the dihydrochloride. The protonation on the nitrogen atoms influenced the surrounding proton positions in their electron density. The loss in resolution hints on an incomplete protonation for both nitrogen sites; an overlapping of signals from non-protonated, once protonated at both positions and twice protonated molecules leads to broader and less sharp

signals. Equivalent measurements on vacuum-dried samples showed the same spectral characteristics. With all three solvents, comparable peak shift changes and additional signals were detected.

The DSC results of the first heating cycle (Figure 4.26, left) showed a small melting signal only for the spray-dried $\cdot 2\text{HCl}$ sample, along with a glass transition temperature at 84 °C. The other samples had similar T_g s in the range of 60–70 °C, with no evidence of melting. The monohydrochloride samples from both spray and vacuum drying showed exothermic events at 115 °C and 154 °C, respectively. In contrast, the second heating cycle for all the samples did not show melting or exothermic transitions (Figure 4.26, right). The dihydrochloride samples showed T_g s in the range of 50–60 °C, while the monohydrochloride forms showed no thermal events.

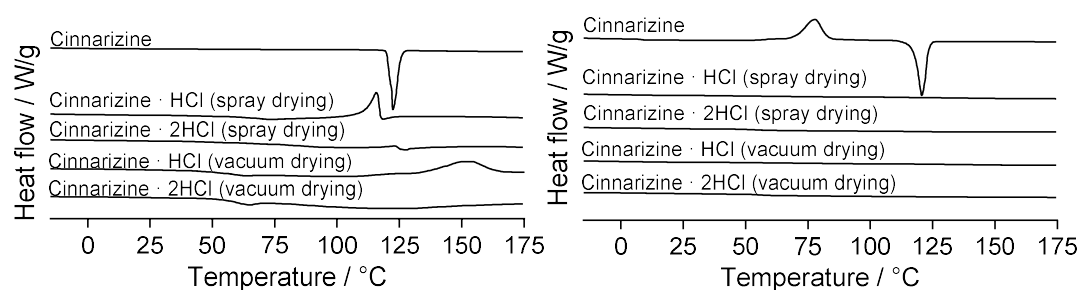


Figure 4.26 DSC thermograms of cinnarizine-HCl salts. Left: first heating in non-hermetic pans. Right: second heating in non-hermetic pans. Exothermic events are plotted upwards. The curves are stacked for better visualisation.

The thermal events are summarised in Table 4.12, together with the results for cinnarizine in both heating cycles. Except for the dihydrochloride prepared by spray drying, no melting peak was detected, indicating that the other samples were all amorphous. The melting peak for the dihydrochloride was at 128.4 °C, which is higher than the melting temperature of cinnarizine, and the peak was small in magnitude, leading to the assumption that the signal was an artefact and not melting of crystalline substance.

Table 4.12 Thermal events in cinnarizine and its HCl-salts prepared by spray drying (SD) and in the vacuum drying cabinet.

		Cinnarizine	Cin·HCl (SD)	Cin·2HCl (SD)	Cin·HCl (vacuum)	Cin·2HCl (vacuum)
First heating	T_g [°C]	-/-	69.1	83.9	59.6	59.9
	T_m [°C]	121.0	-/-	128.4	-/-	-/-
	Exothermal [°C]	-/-	115.8	-/-	154.1	-/-
Second heating	T_g [°C]	7.8	-/-	58.6	-/-	52.4
	T_m [°C]	119.6	-/-	-/-	-/-	-/-
	Exothermal [°C]	89.4 (cryst.)	-/-	-/-	-/-	-/-

XRPD confirmed that the spray-dried materials were amorphous and did not show any evidence of crystallinity (Figure 4.27). Monohydrochloride and dihydrochloride spray-dried samples produced broad halos recognisable as amorphous solids, and there were no characteristic Bragg peaks.

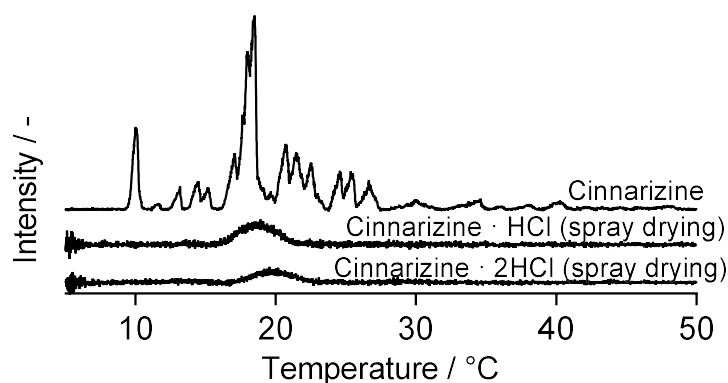


Figure 4.27 XRPD pattern of cinnarizine-HCl salts. The curves are stacked for better visualisation.

The solubility studies notably showed an influence of protonation (see Table 4.13). Both forms behaved similarly, and appeared to have a larger solubility when compared to the non-salt form in aqueous medium, including water and phosphate buffer. The dihydrochloride samples were slightly more soluble than the monohydrochloride samples, which could be due to an increased percentage of protonation and therefore an increased ionisation, though the differences were not statistically significant. The octanol solubility was lower for both salts compared to the pure form, as expected from their increased polarity. They showed approximately the same octanol solubility, although the dihydrochloride had a larger variation between replicates.

Table 4.13 Thermodynamic saturation solubilities of cinnarizine and its hydrochlorides prepared by spray drying.

	H ₂ O	Phosphate buffer pH 6.8	Octanol
Cinnarizine	0.43 ± 0.33 µg/mL	0.25 ± 0.00 µg/mL	17.7 ± 2.1 mg/mL
Cinnarizine hydrochloride	1.26 ± 0.16 mg/mL	0.71 ± 0.01 µg/mL	3.68 ± 0.16 mg/mL
Cinnarizine dihydrochloride	1.54 ± 0.30 mg/mL	0.80 ± 0.24 µg/mL	4.22 ± 0.85 mg/mL

4.2.11 Solubility parameters

Hildebrand solubility parameters were obtained by computational modelling and are listed in Table 4.2 for the APIs and polymers. Table 4.14 gives the absolute values of the differences between solubility parameters of the polymers and cinnarizine, respectively. The comparison of solubility parameters is a rudimentary model. Though solvents themselves can be characterised with the solubility parameters, their potential presence is not taken into account when comparing the parameters of two substances as, in this case, polymer and drug. Consequently, it provides merely a basic understanding of interactions within a water-free system.

Table 4.14 Differences between the solubility parameters for cinnarizine and the selected polymers. Given is the absolute value of the difference. The overall solubility parameter for cinnarizine is 19.14, the van der Waals solubility parameter is 18.10, and the electrostatic solubility parameter is 6.22.

	$\Delta\delta_{total}$ [MPa ^{1/2}]	$\Delta\delta_{vdw}$ [MPa ^{1/2}]	$\Delta\delta_e$ [MPa ^{1/2}]
MAE-A	4.83	7.33	3.19
EPO	6.11	5.91	1.60
HPMCAS	1.70	5.23	5.54
HPMC	1.45	4.99	5.67
VA64	4.20	4.50	0.05
K30	3.93	4.29	0.15
PAA	0.74	5.27	8.97
PSSA	0.19	5.36	7.80
Good (0 – 2)	Fair (2 – 5)		Poor (> 5)

The overall differences between the solubility parameters were considered good or fair, with the exception of the comparison with EPO. A more detailed analysis revealed that PAA and PSSA demonstrated the smallest differences, followed by HPMCAS and HPMC. In the separated analyses of both $\Delta\delta_{vdw}$ and $\Delta\delta_e$, all polymers exhibited substantially large deviations from those of cinnarizine. This finding indicates that cinnarizine is characterised by poor miscibility and dissolution properties in the polymers. It is noteworthy that for the highly polar polymers PAA and PSSA, the $\Delta\delta_{vdw}$ is smaller than $\Delta\delta_e$, and only in the overall evaluation, they demonstrate compatibility with cinnarizine. It appears that Coulomb forces are not beneficial in this instance, given that cinnarizine is characterised by its high degree of non-polarity and absence of charge. As the solubility parameters were calculated for the pure substances in their neutral form, they do not account for possible proton exchanges and changes in the molecular charges. It is hypothesised that these exchanges and charges might add a benefit to the interactions and therefore the miscibility. HPMCAS and HPMC were found to be more polar than cinnarizine. However, a substantial degree of compatibility was observed, attributable to the presence of extensive unpolar domains within the celluloses. The combination of cinnarizine with the celluloses has been shown to yield a small $\Delta\delta_{total}$, which can be attributed to the beneficial balance between potential hydrogen bonding and non-polar interactions.

Surprisingly, for the combination cinnarizine-EPO a $\Delta\delta_e$ of only 1.60 was calculated. It is evident that the molecules appear to be comparable regarding their inability to engage in Coulomb interactions with their own species. Both molecular moieties cannot form hydrogen bonds with themselves. Consequently, the potential for interactions between EPO and cinnarizine is limited. Interactions between these two components are primarily driven by van der Waals forces. Molecular dynamics simulation revealed a modest number of aromatic hydrogen bonds (see Chapter 4.2.13); however, the overall number of interactions remains limited. In the context of MAE-A, the $\Delta\delta_{total}$ of 4.83 could be classified as fair, mainly caused by the Coulomb component of the equation (δ_p^2 in Eq. (1.7)).

The investigation revealed that the VA64 and K30 models exhibited optimal $\Delta\delta_e$ values. Nevertheless, this cannot compensate for larger differences in the non-polar part of the overall solubility parameters, leading to fair values of 4.20 and 3.93, respectively. It was expected that the $\Delta\delta_{vdW}$ would also demonstrate a high degree of comparability between cinnarizine and the polymers. The beneficial effect in stabilising cinnarizine, as evidenced by molecular interactions, is likely resulting from the optimal ratio of polar and non-polar interactions. Although VA64 and K30 can only form aromatic hydrogen bonds with cinnarizine, comparable to EPO, the overall effect appears to be beneficial here.

For the total solubility parameter differences, the ranking of the polymers was found to be PSSA < PAA < HPMC < HPMCAS < K30 < VA64 < MAE-A < EPO.

4.2.12 pK_a calculations

For cinnarizine, the two protonatable positions on the nitrogen atoms in the piperazine moiety demonstrate almost identical basicity and can be classified as weak bases, with pK_a values of 6.39 and 6.40 (for the chemical structure, see Figure 3.1). Upon the protonation of one site, the pK_a of the second nitrogen atom decreases, thereby rendering it a stronger acid and thus decreasing its tendency to undergo protonation at a neutral pH (pK_a of 2.13 and 4.33 if other nitrogen atom is protonated already, see Table 4.3). This phenomenon is illustrated in the bar chart (Figure 4.28, right): at a pH of 6.8, the probability of both sites being protonated is 0%, whereas approximately 30% of the molecules are singly protonated at each position, and 43.5% are fully deprotonated. The shown plot for trimethoprim is discussed later in chapter 4.3.16.

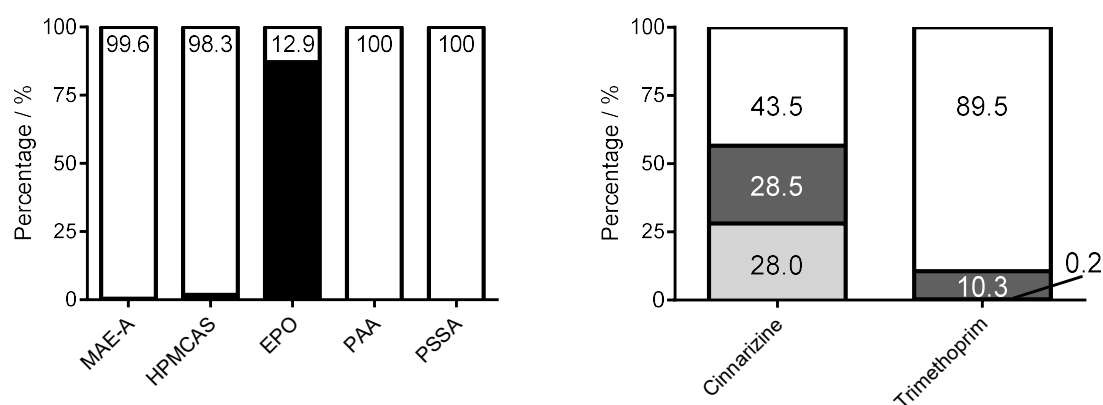


Figure 4.28 Distribution of protonated and deprotonated molecules at pH 6.8 for polymers (left) and active substances (right). Left: Protonated species are shown in black; the numbers give the percentage of deprotonated molecules. Right: Light-grey bars represent molecules with only position 1 protonated, dark-grey bars represent molecules with only position 2 protonated and white bars indicate a deprotonated molecule. The numbers show the respective percentages. Cinnarizine and trimethoprim with both positions protonated have a probability of zero percent at pH 6.8.

It has been demonstrated that the ionisation state of the compounds plays a critical role in their solubility in aqueous media, as charged (ionised) molecules are generally more water-soluble. MAE-A, HPMCAS, PAA, and PSSA, being acidic in nature, are almost fully deprotonated at the

tested pH of 6.8. This means that they predominantly exist in their ionised (anionic) forms and consequently, it is expected that they will exhibit a good aqueous solubility. EPO, a weakly basic polymer, is almost 90% protonated, meaning it exists predominantly in its charged (cationic) form. The modelled pK_a of 7.63 fits to the range found in literature, where it is given as 7.6 [167] or 8.5 [168], respectively. However, the difference between pK_a and pH is less than 1 unit, whereas a ± 2 unit difference is necessary to ensure that >99% of the molecules are ionised, thus significantly improving solubility. Despite its apparent ionisation, EPO is not soluble at pH 6.80, in line with experimental findings.

With regard to the API, cinnarizine demonstrates approximately 60% protonation at one of the two protonatable sites at pH 6.8, a value insufficient to obtain a notable improvement in solubility, given the proximity of the pH to its pK_a . Despite the fact that this partial protonation does not enhance solubility to a significant degree, it could still enable interactions with acidic excipients that are deprotonated, particularly in buffered systems where the pH remains stable.

4.2.13 Molecular modelling

In molecular modelling studies, cinnarizine was combined with selected polymers in drug loads of 10%, 30%, 50%, 70%, and 90% (w/w). The number of interaction events was counted.

For cells created with cinnarizine, the maximum number of hydrogen bonds between the API and the respective polymer was found in a range between 30% and 50% drug loading in all cases (see Figure 4.29; aliphatic and aromatic hydrogen bonds were summed up). As expected, most hydrogen bonds were detected in the simulations with PAA and PSSA, followed by MAE-A and HPMCAS. The counts for HPMC, VA64 and K30 were comparable to those of MAE-A and HPMCAS for low and high drug loads but were slightly lower in the maximum area around 50% drug load. It is important to note that for VA64 and K30, only aromatic hydrogen bonds were identified. A similar outcome was observed in the simulations with EPO, where the lowest interaction count was noted for all drug loads. The total count of hydrogen bonds increased by more than 100% when comparing EPO to PAA. When examining hydrogen bonds exclusively for aliphatic or aromatic ones, the rankings remained consistent, with the exception of VA64, K30 and EPO, which did not exhibit aliphatic hydrogen bonds, as previously discussed.

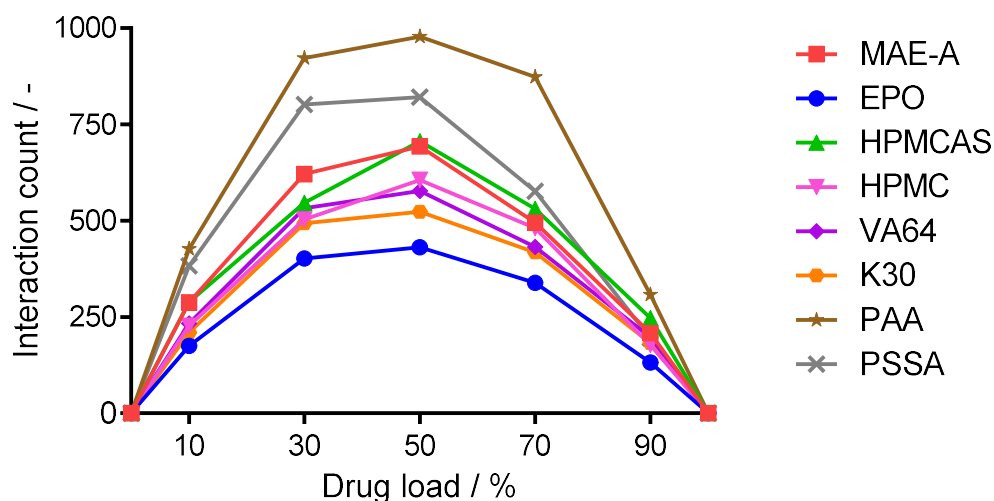


Figure 4.29 Interaction counts for cinnarizine-polymer combinations including aliphatic and aromatic hydrogen bonds. The polymers shown are MAE-A (red squares), EPO (blue circles), HPMCAS (green triangles), HPMC (pink inverted triangles), VA64 (purple rhombuses), K30 (orange hexagons), PAA (brown stars) and PSSA (grey crosses).

A detailed examination of the individual systems reveals a comparison between the total number of countable interactions (including all types of hydrogen bonding and π - π -stacking) between the API and the polymer molecules with the ones between the API molecules alone. This is demonstrated in Figure 4.30, in which the API-API curves are comparable for all the tested systems. This shows that disruptions to API-API-interactions are predominantly influenced by the percentage of interfering polymer molecules, rather than the polymer's type, molecular size, or functional groups.

The peak of the API-polymer-interaction curve was found to be the most pronounced for PAA and PSSA again, followed by MAE-A. The performance of EPO was found to be poorest and was comparable to the performance when only hydrogen bonds were observed. HPMCAS, HPMC, VA64, and K30 all exhibited similar total interaction counts. The area under the curve was derived using the trapezoidal rule and is shown in Figure 4.31. As demonstrated in the bar chart, when the entire range from zero to 100% drug load was utilised, the ranking sequence was as follows: PSSA > PAA > MAE-A > HPMCAS > HPMC > VA64 > K30 > EPO. It is evident that there were only minor differences between the systems.

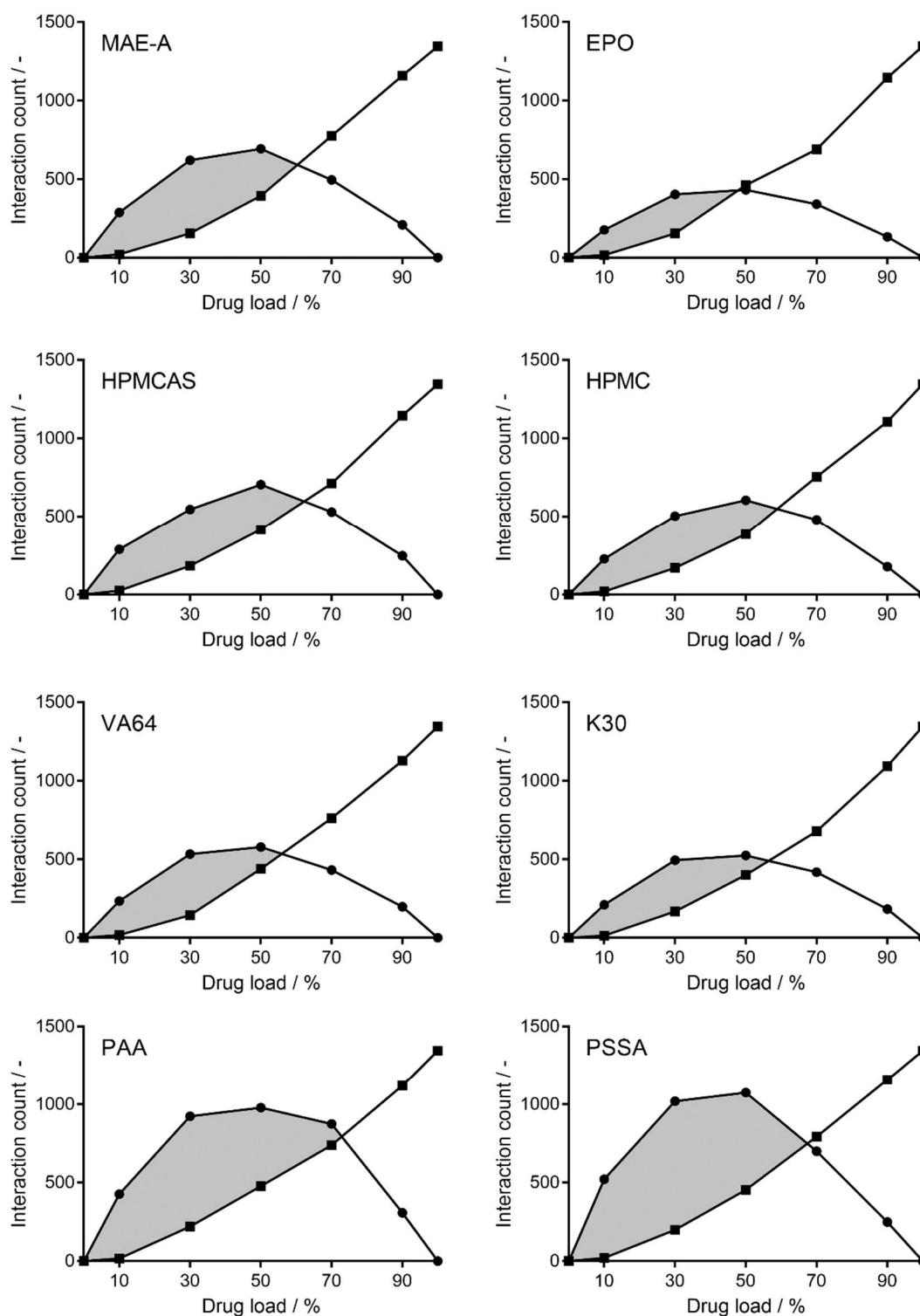


Figure 4.30 Combined interaction counts (hydrogen bonding + π - π -stacking) for cinnarizine-polymer combinations with MAE-A, EPO, HPMCAS, HPMC, VA64, K30, PAA, and PSSA. Shown is the number of API-polymer-interactions (circles) and API-API-interactions (squares); the grey area marks the range where API-polymer-interactions outnumber API-API-interactions.

Additionally, the area between the curves was calculated. The area in question is denoted by the grey hatching in the plots. The area between the curves is indicative of the calculation of the total

interactions between API and polymer, as well as the API-API interactions. It not only shows how many interactions there are between API and polymer, but also by how much these exceed the API-API interactions. The area represents the total range in which the API-polymer interactions exceed the API-API interactions in number. Here, the ranking corresponded to that of the entire curve, albeit with certain varied ratios. Figure 4.31 also shows the drug load at which the two curves intersect. This point of intersection does not provide a maximum drug loading for a homogeneous system; however, it may offer insights into the stability and compatibility of such systems. In the case of PAA, the curves intercept at the highest drug load with more than 70%, while for EPO, the intercept is below 50% drug load. The remaining systems vary in-between those limits, again in the anticipated order. The maximum possible drug load cannot be derived from the intercept as the molecular modelling assumes a perfectly mixed system from the outset, with well-defined numbers of molecules and parameters regarding the physical conditions, such as pressure and temperature. While this is not transferable to a laboratory experiment on a one-to-one basis, the applied methodology is a valuable tool in the identification of polymer candidates that demonstrate superior functionality when compared with others.

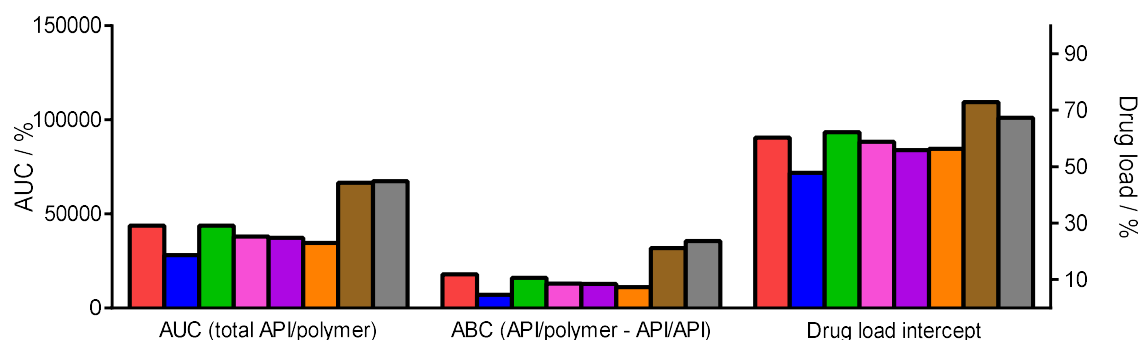


Figure 4.31 Area under the curve (AUC), area between the curves (ABC) and drug load intercept for API-polymer systems with cinnarizine. Shown are results for MAE-A (red), EPO (blue) HPMCAS (green), HPMC (pink), VA64 (purple), K30 (orange), PAA (brown), and PSSA (grey), calculated from the curves in Figure 4.30.

In addition to cells with different drug loads, a molecular dynamics simulation was performed with 500 molecules of cinnarizine and one molecule of each polymer, respectively. The investigation was conducted with the objective of ascertaining the number of possible interactions per polymer molecule, given the presence of an excess of API molecules. The polymer size was scaled down as described in Table 3.8. For all structures, sizes that were comparable were utilised. Furthermore, the number of identified interactions was divided by the number of monomers, rendering it independent of the chosen molecule size. The total number of interactions is comprised of aliphatic and aromatic hydrogen bonds as well as π - π -stacking. These interactions are not weighted according to their respective strength. In Table 4.15, the results for cinnarizine are summarised.

Table 4.15 Interaction count per monomer obtained in “500+1”-experiments for cinnarizine with different polymers.

	MAE-A	EPO	HPMCAS	HPMC	VA64	K30	PAA	PSSA
Interactions per molecule	103	20	107	60	35	28	115	81
Interactions per monomer	0.55	0.50	1.37	1.58	0.61	0.64	0.83	1.98
Interactions per real molecule (multiplied by downsizing factor)	2060	200	107	600	350	280	1150	810
Interactions $\cdot 10^{21}$ per g of polymer	3.86	2.55	3.78	4.29	3.65	3.43	6.90	6.48

The total number of interactions for one molecule of polymer decreases in the order PAA > HPMCAS > MAE-A > PSSA > HPMC > VA64 > K30 > EPO for cinnarizine. This finding is largely consistent with the results previously discussed using molecular dynamics on larger cells, except for the performance of PSSA. When the number of interactions is divided by the number of monomers, PSSA has the highest number of interactions with 1.98. This demonstrates the high potential of utilising PSSA with an increasing number of molecules. It is advantageous for the monomer unit to possess a low molecular weight, thereby ensuring a high number of monomers per molecule. This is exemplified by the case of PAA, where the number of interactions per monomer is average, but due to the high number of monomers, the total count of interactions is high. Conversely, HPMC exhibits the second highest number of interactions per monomer, yet the significant size of its monomer units results in a total interaction count in the lower area. The number of interactions per monomer unit decreases in the following order: PSSA > HPMC > HPMCAS > PAA > K30 > VA64 > MAE-A > EPO.

When the different downsizing factors are considered, by far most interactions were found for MAE-A and least for HPMCAS. The other ranking remains the same as for the modelled molecules, as they were decreased in size by the same factor. With the molar mass and the Avogadro constant, the theoretical interactions per 1 g of polymer with an excess of cinnarizine molecules was estimated. The results are listed in Table 4.15, divided by 10^{21} for the sake of clear visualisation. By this, HPMCAS interactions outnumber the ones in VA64, K30, and EPO due to its low molecular weight and therefore the higher number of molecules in 1 g of polymer substance. With this calculation, by far most interactions were found for PAA and PSSA. While the reference to mass is the most relevant calculation, given that the formulation is also based on mass instead of molecule number in the laboratory, it is essential to note that these calculations are purely theoretical and only provide an estimate of the polymer sequence. The actual number of interactions is dependent on numerous factors, including temperature, density, humidity, and the ratio of polymer to active ingredient molecules.

4.2.14 Summary for cinnarizine formulations

This study on the ability of polymers to form stable ASDs through different preparation methods, such as melt quenching, HME and VCM, as well as the investigation on the underlying molecular interactions, reveals not only critical insights into the interplay between polymer characteristics, drug load and processing method, but also their implications for long-term stability and dissolution performance. The rankings that were found for the performance of the polymers are summarised in Table 4.16. The polymers are listed from the best performance to the worst for the tested methods. It can be seen that in the solid-state experiments regarding the manufacture of ASDs, residual crystallinity, long-term stability, limits and phase diagrams and deviations from Gordon-Taylor and Flory-Huggins, the acidic polymers MAE-A and HPMCAS performed mainly as expected.

Based on the classification system by Baird, van Eerdenbrugh and Taylor [59], cinnarizine can be classified as class II substance with an intermediate crystallisation behaviour. In DSC analysis, no crystallisation is observed during rapid cooling of the melt, but the amorphous state is not stable upon reheating. With addition of polymers, the resistance to crystallisation could be enhanced, mostly by using acidic polymers. For HME samples, MAE-A and HPMCAS were similar in their ability to stabilise the amorphous state, both short- and long-term. In melt quenching, the performance of MAE-A was surprisingly poor. The lack of mechanical shear forces and mixing could have led to an insufficient distribution of cinnarizine in the polymer. Due to its low degradation temperature, MAE-A could only be heated to 160 °C. At this temperature, MAE-A still possesses a high melt viscosity. Contrarily, other polymers were heated to higher temperatures and showed a greater difference to their T_g . This explains also the surprisingly good performance of EPO in melt quenching compared to the other methods. Due to the low T_g of EPO of 45 °C, the molecular mobility of the polymer was high at the melting point of cinnarizine at 120 °C. The T_g of the other tested polymers were near or even above the melting temperature, so that molecular mobility and the possibility to evenly distribute the API in the matrix were lower. The same was found by Trivino et al. in a study with flutamide and the polymers EPO, HPMC, povidone K90 and polyethylene glycol 8,000 [33]. When surpassing the T_g , the viscosity decreases and molecular mobility increases rapidly. It was suggested that zero molecular mobility can be assumed only at temperatures of 50 °C below the T_g of a system [32]. It is assumed that for polymers with a low T_g such as EPO, the beneficial low viscosity at the melting temperature of the API prevails the lack of possible molecular interactions with a weak base as cinnarizine. In contrast, the presence of interactions becomes more important in the liquid state, for example in dissolution testing. For EPO, strong molecular interactions were found in a study by Meng et al. with curcumin as weakly acidic drug substance [40]. Here, povidone was inferior to the alkaline polymer EPO, which led to the highest dissolution rate of the active substance. For an alkaline active substance, such effects of EPO are missing.

Table 4.16 Overview of polymer rankings according to the methods applied in this study for cinnarizine. Beneficial effects or performances regarding the respective characteristic decrease from left to right (such as lower degree of drug crystallisation, higher amount of dissolved drug, and more possible drug-polymer interactions). The colours indicate the type of highlighted functional groups: acidic (grey), neutral (white), basic (black); if two polymers of different groups performed similar, they are marked as light grey.

		Beneficial effects					
Residual crystal- linity	50% ASDs melt quenching	HPMCAS	EPO	VA64	HPMC	MAE-A	K30
	50% ASDs HME	HPMCAS = MAE-A		HPMC	EPO	K30	VA64
	50% ASDs HME after 18 months 25 °C / 60% r.h.	HPMCAS = MAE-A		VA64	HPMC	K30	EPO
	50% ASDs HME after 18 months 40 °C / 75% r.h.	HPMCAS = MAE-A		K30	VA64		HPMC
	50% ASDs SD	MAE-A	VA64	HPMCAS	HPMC	K30	
Phase dia- grams	Limit of drug load to be kinetically stabilised	MAE-A	VA64	HPMCAS = K30		HPMC	EPO
Gordon-Taylor	Δk theoretical-fitted equation	MAE-A	VA64	HPMCAS	K30	HPMC	EPO
	ΔAUC theoretical-fitted equation	VA64	MAE-A	HPMCAS	K30	HPMC	EPO
Flory-Huggins	χ	HPMCAS	EPO	MAE-A	K30	HPMC	VA64
Solubility increase	1% polymer addition	K30	EPO	MAE-A	HPMC	HPMCAS	VA64
	5% polymer addition	MAE-A	HPMC	K30	EPO	VA64	HPMCAS
Dissolution	AUC 10% milled extrudates (FaSSiF)	MAE-A	K30	HPMC	VA64	HPMCAS	EPO
	AUC 50% milled extrudates (FaSSiF)	HPMC	K30	MAE-A	HPMCAS	VA64	EPO
	AUC both drug loads VCM (FaSSiF)	VA64	K30	HPMC	MAE-A	EPO	HPMCAS
	Dissolution speed of polymers	VA64	K30	HPMC	MAE-A	HPMCAS	EPO
1H-NMR	δ_{max}	MAE-A	HPMCAS	K30	VA64	HPMC	EPO
Modeling	$\Delta\delta_{total}$ (solubility parameter)	HPMC	HPMCAS	K30	VA64	MAE-A	EPO
	AUC (total API/polymer)	MAE-A	HPMCAS	HPMC	VA64	K30	EPO
	ABC (API/polymer – API/API)	MAE-A	HPMCAS	HPMC	VA64	K30	EPO
	No. interactions per monomer	HPMC	HPMCAS	K30	VA64	MAE-A	EPO
	No. interactions per molecule; calculated for real size	MAE-A	HPMC	VA64	K30	EPO	HPMCAS
	No. interactions per 1 g of substance	HPMC	MAE-A	HPMCAS	VA64	K30	EPO

The results obtained by the phase diagrams fit largely to the performances in melt quenching and hot melt extrusion. Besides the T_g , the solubility of the API in the polymer correlates with the ability of a polymer to stabilise the amorphous form and influences the limits of the single phases. Here, the maximum amount of cinnarizine that could be kinetically stabilised was highest with MAE-A,

followed by VA64 and then HPMCAS and K30, where the results were similar. A ranking of $\text{HPMCAS} < \text{VA64} < \text{povidone}$ was found in a study with acetaminophen and naproxen, showing that povidone performed best for both a neutral and an acidic drug substance [57]. The same study showed that with HPMCAS, the acidic naproxen formed stronger interactions than acetaminophen. In a study with nifedipine ASDs, it was found that povidone formed stronger molecular interactions with the API than HPMCAS, thereby reducing the mobility and fostering physical stability [66]. Cinnarizine as a weak base does not benefit from povidone as excipient to the same extent as neutral and acidic APIs in the different studies.

It is assumed that hydrogen bonds between drug and polymer are weaker than these between two or more API molecules by self-association when the T_g values are deviating negatively from the predicted values by Gordon-Taylor [53]. In these experiments, MAE-A and HPMCAS induced the most interactions as seen by the highest Δk and ΔAUC , but also VA64 performed surprisingly well. A detailed study on VA64 revealed that the degree of molecular interactions and the physical stability of ASDs depended on whether the drug molecule is a hydrogen bond donor or not and on the crystallisation behaviour of the API alone [73]. The importance of hydrogen bond interactions in the stabilisation by VA64 was also underlined in a recent study on the availability of the carboxyl group of the active by chemical shielding and steric hindrance [169].

Dissolution experiments led to results that were not expected from the previous findings. Referring to the polymer type, the potential for acid-base interactions did not govern the performance of the polymers. Instead, the dissolution speed of the polymers was crucial for the overall performance, independent of drug load and geometrical shape.

With VA64 and K30, the supersaturation level was highest, but also precipitation occurred, so that the high level of drug concentration could not be stabilised over the tested period of four hours. Povidone as well as other highly hydrophilic polymers are known to facilitate rapid dissolution and high levels of supersaturation [27]. Nevertheless, the stabilisation of the supersaturated state is better supported by polymers with a balance between hydrophilicity and hydrophobicity as HPMCAS [61]. For HPMCAS, the grade is also influencing the dissolution behaviour due to the varying opening pH-values based on different substituent patterns. HPMCAS with an opening pH of 6.5 as used in this study was shown to release drug substances slower than other HPMCAS grades in a study by Kokott et al. [17].

Having a closer look at the geometrical shape of the formulations, the main reason for the better performance of the milled extrudates compared to VCM discs lies in the increased specific surface area. The particle size distributions of the milled extrudates are shown in Figure 8.12 in the appendix. Though the specific surface area of the milled extrudates is higher compared to the discs, there were no substantial differences in the size distributions between the milled extrudate formulations themselves. The maximum of the relative amount q_0 in the particle size distribution was below 10 μm

for all formulations. Therefore, differences in dissolution behaviour within the milled extrudates are unlikely to be based on particle size effects, and particle size is not considered a confounding factor in the interpretation of the data.

Additionally, differences in the homogeneity of API distribution within the ASDs could also lead to poor dissolution kinetics from the VCM discs. A study by Pöstges et al. showed that VCM led to heterogeneous ASDs whilst HME could form homogeneous ASDs with multiple polymers. This finding might be transferable to the homogeneity of APIs in polymers as well [22]. A study by Chen et al. concluded that povidone, when co-dissolving from an ASD with a drug molecule, can stabilise a supersaturated state of the drug, whilst it is not the case when povidone was pre-dissolved in the medium prior to API addition [26]. The authors concluded that pre-dissolved polymers were hindered to form interactions with the API due to the existing interactions with water molecules. For HPMCAS, which formed hydrophobic interactions with the API, the timing of polymer dissolution was not critical for the beneficial effect on drug dissolution. The pre-dissolving of the polymer represents an extreme case of an ASD with inhomogeneous distribution of drug and polymer molecules, which can dissolve independently.

Regarding the drug load, influences were only found for systems with an overall good performance at 10% (w/w) API content. The residual crystallinity in higher loaded ASDs could cause a lower level or even a lack of supersaturation. Additionally, the presence of residual crystals could enhance recrystallisation in the dissolution medium and therefore a sharp decrease in drug concentration appears. With lower drug load, the concentration of available polymer for the stabilisation of a supersaturated state in the medium and the amorphous form in the solid particles is higher. All these factors led to the observed results, in which the dissolved amount from 50% (w/w) samples was lower compared to the 10% (w/w) samples. Previous studies on the influence of drug load on the dissolution behaviour in ASDs revealed that there appears to be a cut-off above which the dissolution rate drastically decreases [16,159]. The so-called congruent release describes the regime in which the dissolution rate of drug and polymer are similar and the release is driven by polymer characteristics. Above a certain limit, which is different for each drug-polymer system, the higher loading of API leads to a change in dissolution behaviour. When the API is controlling the dissolution behaviour, a decrease in drug release rate is observed [34–36]. This is consistent with the findings in this work.

Spray drying resulted in all cases in systems with higher residual crystallinity compared to hot melt extrusion, except for the ternary systems. In spray drying, no completely amorphous system could be manufactured. Still, the higher crystallinity did not necessarily lead to a reduced drug dissolution. The factor of the smaller particle size was found to be more relevant in enhancing dissolution for MAE-A systems when comparing spray-dried samples to melt extruded samples (50% (w/w) drug load).

Ternary systems were not found to be beneficial compared to their respective binary counterparts, both in stabilising the amorphous solid state and in increasing the dissolution rate. The residual crystallinity was higher compared to the binary system with at least one of the incorporated polymers and dissolution rates were not only lower, but also with larger fluctuations.

The results from NMR titrations showed a clear ranking of acidic > neutral > basic for the selected polymers in forming interactions with cinnarizine. Though the acidic polymers were superior, there were also effects observed for the neutral polymers, restricting the assumption that the effects were only pH dependent. Besides this study, results on ¹H-NMR with ASDs are limited. As mentioned before, ¹H-NMR was employed in indomethacin ASDs to investigate molecular interactions on a qualitative basis by Prasad et al. [23]. Here, a concentration dependent shielding of the proton in the carboxylic acid group by the presence of povidone K90 was found. This was attributed to an interaction that can also influence the dissolution behaviour. In the present work, most interactions were found neighbouring the basic nitrogen with least steric hindrance. This shows that not only the basicity or acidity of a substance is crucial for potential interactions, but also the steric surrounding and accessibility thereof. Especially for hydrogen bonding and salt bridges, there needs to be an atomic distance below 4 Ångström, while for van der Waals forces, an approximation of 5 – 6 Ångström is sufficient [104].

Molecular modelling revealed that the order of polymers with most interactions is highly dependent on the way of counting the interactions, meaning if they are referring to the number per monomer unit, the number per molecule or the number per a fixed mass. Regarding the interactions per 1 g of substance, the expectations of MAE-A and HPMCAS being superior to VA64 and K30, followed by EPO with least interactions were fulfilled. The high number of interaction sites in HPMC molecules were unexpected, but can be related to the large hydrophobic moieties interacting with the lipophilic molecule cinnarizine.

Overall, MAE-A and HPMCAS provided the most robust amorphous stabilisation of cinnarizine, whereas VA64 and K30 achieved the highest supersaturation in dissolution testing governed by the polymer dissolution speed instead of molecular interactions. Supporting these observations, molecular modelling and NMR-titrations showed stronger drug-polymer interactions for acidic polymers compared to neutral and basic ones. Thus, no single polymer excelled in all criteria, highlighting the necessity of balancing stability and dissolution to achieve optimal ASD performance.

4.3 Trimethoprim

Essential contents of this chapter are based on the research article manuscript “Molecular modelling and experimental investigations into the process-related embedding and dissolution profiles of trimethoprim amorphous solid dispersions”, by Selina Schmidt, Konrad Brachmann, Luca Esser, Justin Duong, Jörg Breitzkreutz and Heiko A. Schiffter; Target Journal: Journal of Drug Delivery Science and Technology (2026), which has been prepared and submitted to the target journal.

4.3.1 Crystalline trimethoprim

Crystalline trimethoprim was processed by hot melt extrusion, spray drying, melt quenching and milling. Only the samples obtained from milling showed a recrystallisation signal at 85 °C in the DSC analysis during the first heating cycle, suggesting that only a part of the API was in an amorphous state (see Figure 4.32, left). The calculated residual crystallinity in the sample was $94.9 \pm 4.4\%$, whilst for the graphs obtained from the other methods, the AUC of the melting enthalpy was equal to that of unprocessed trimethoprim. Still, for the milled trimethoprim, no glass transition temperature could be measured due to the small portion of amorphous substance. The T_g of trimethoprim could only be obtained by melt quenching with a change in the heating rate. When reducing the rate from 10 K/min to 2 K/min, a glass transition signal was detected and also a recrystallisation peak was observed afterwards.

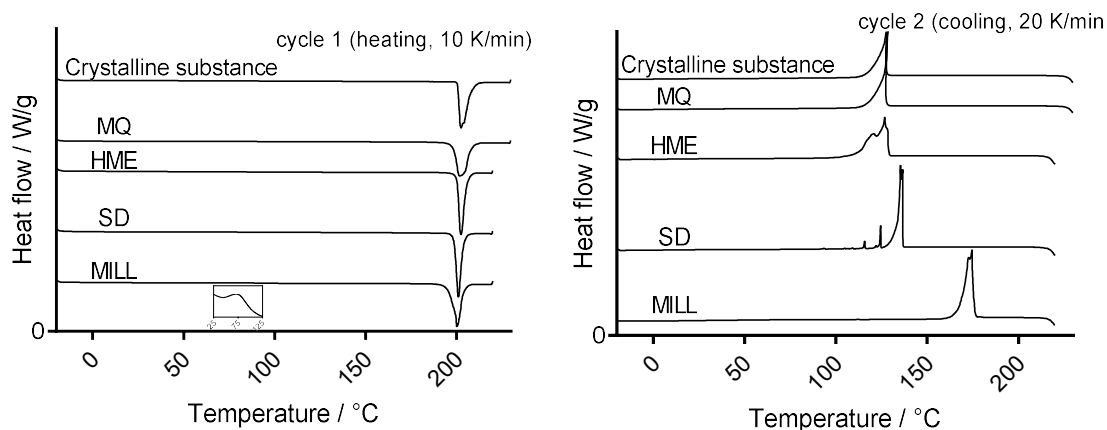


Figure 4.32 DSC analysis of trimethoprim as crystalline substance and processed by melt quenching, hot melt extrusion, spray drying and ball milling. The left graph shows the first heating cycle at 10 K/min, the right graph shows the rapid cooling at 20 K/min. Exothermal events are plotted upwards.

During rapid cooling, all samples showed recrystallisation at temperatures above 100 °C as seen in Figure 4.32, right. For the milled samples, the recrystallisation temperature was highest with more than 150 °C. The peak shape, especially in the unprocessed API, implied a rapid recrystallisation at a specific temperature instead of a broader temperature range. The spray-dried samples showed multiple signals, presumably due to the residual solvent left in the hermetically sealed pans. In all cases, the recrystallisation during cooling was complete, meaning that at room temperature the

samples were exclusively crystalline. With that behaviour, trimethoprim can be classified as class I substance according to Baird, van Eerdenbrugh and Taylor [59].

The XRPD analysis of the differently processed samples revealed that the main peaks were at the same two-theta position (Figure 4.33). Still, the signal shape and intensity were not consistent. Interestingly, spray drying and milling led to almost identical patterns, though milling was performed solvent-free. The spray-dried sample and the one obtained from vacuum drying, which both were manufactured with methanol, differed from each other in the strength of the signal at 26 ° and in the presence of the one at 35 °. The extruded trimethoprim only varied from the unprocessed API by the magnitude of the signal at 17 °.

In literature, four different polymorphs are known for trimethoprim, with the commercially available form I being the most stable. During heating, forms II, III and IV transform into form I either directly or in two steps (form III transforms to form II and then to form I). Though XRPD-patterns have been published for all polymorphic forms, they do not fit to the patterns found in this study [170,171]. This suggests that no pure forms were created, but a mixture of two or more forms coexisted. The findings from the DSC analysis, in which no signals for a polymorphic transformation were found, support this hypothesis, as only traces of other forms may be present in the samples and the transformation signals were therefore not strong enough to be detected.

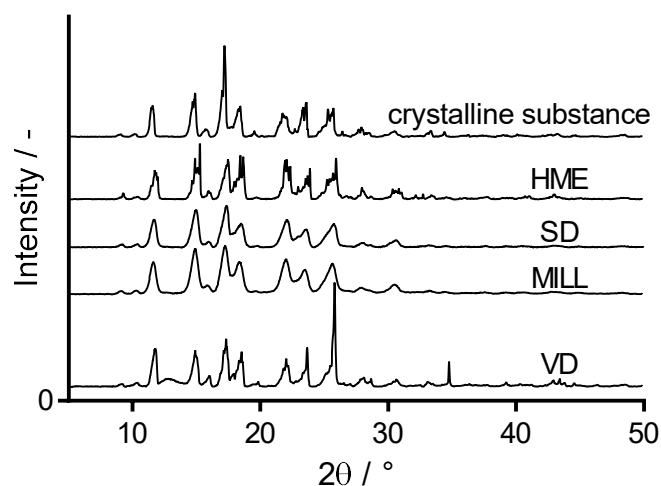


Figure 4.33 XRPD analysis of trimethoprim as crystalline substance and processed by hot melt extrusion, spray drying, milling and vacuum drying.

4.3.2 Melt quenching

The potential to form and stabilise an amorphous state of trimethoprim in the polymers was expected to be highest in melt quenching. Here, the ASDs were formed by rapid cooling upon melting of the substances in the DSC and the analysis was performed directly afterwards by reheating the pan. No influences of storage, transfer, dwell time, residual solvent or other factors should interfere

with the system. Due to the high melting temperature of trimethoprim which had to be reached, the glass transition temperatures of the polymers were surpassed to a large extent. MAE-A and K30 could therefore not be used in the melt quenching studies, as the melting point of trimethoprim was higher than their degradation temperatures. Though the mechanical forces in hot melt extrusion were found to be beneficial in some cases with cinnarizine, these were limited to high-viscosity polymers as MAE-A where an active mixing was required for embedding. Therefore, the results of embedding capacity in melt quenching gave the upper limit up to which drug load the polymers were suitable for manufacturing ASDs with trimethoprim solvent-free.

Table 4.17 Summary of the residual crystallinity of trimethoprim amorphous solid dispersions. Numbers in red boxes indicate the degree of crystallinity relative to the whole system in percent as determined by the melting enthalpy in DSC (mean of n = 3). Green boxes indicate amorphous systems without residual crystallinity. The corresponding DSC plots are shown in Figure 8.13 in the appendix.

Polymer	Drug load / %	Hot melt extrusion	Spray drying	Melt quenching
EPO	10	2.3	-/-	3.5
	30	12.6	5.2	4.9
	50	41.0	24.9	31.0
HPMCAS	10		-/-	
	30		13.4	
	50	4.3	19.7	
	50 (90 °C in SD)	-/-	10.5	-/-
	70	-/-	34.1	5.5
HPMC	10	3.4	-/-	
	30	4.1	19.0	
	50	3.7	25.5	
	50 (90 °C in SD)	-/-	18.0	-/-
	70	20.0	40.0	16.9
VA64	10	3.7	-/-	
	30	5.2	6.7	2.2
	50	21.9	14.5	3.2
	70	22.8	34.9	7.0
HPMCAS / VA64 1:1	30		-/-	-/-
	50	2.3	-/-	-/-
	70	20.1	-/-	-/-

As summarised in Table 4.17, melt quenching led to the highest level of possible amorphous drug load for HPMCAS, HPMC, and VA64. Consistent with the literature on basic APIs [47], HPMCAS exhibited the strongest stabilisation: fully amorphous systems were achieved up to 50% drug load, and at 70% only 6% residual crystallinity remained. HPMC matched the 50% amorphous limit but showed 17% crystallinity at 70%. A notable distinction emerged during reheating: HPMCAS samples at 10-50% displayed no endo- or exothermal events, whereas HPMC at 30% and 50% showed equal magnitudes of crystallisation and melting peaks, indicating that although these systems were amorphous before heating, the thermal input induced instability and recrystallisation at 130-150 °C. The superior physical stability of HPMCAS with basic APIs is well established and attributed to a combination of acid-base and hydrogen bonding interactions, hydrophobic

associations, and disruption of drug-drug contacts [26,172–174]. VA64 supported amorphous formation at 10% and was nearly amorphous at 30% (2% crystallinity). All EPO-samples were partly crystalline. The complete data on DSC-plots are listed in the appendix (Figure 8.15, Figure 8.17, Figure 8.18, Figure 8.19, and Figure 8.20).

4.3.3 Hot melt extrusion

Trimethoprim was extruded with different polymers at varying drug loads (10%, 30%, 50% and in some cases (HPMC, VA64, HPMCAS / VA64 1:1) 70% w/w) to assess up to which amount of API an amorphous solid dispersion without crystalline parts could be manufactured. The determined content for the single formulations is listed in Table 8.1 in the appendix. Table 4.17 shows the embedding capabilities summarised for hot melt extrusion, spray drying and melt quenching. In all cases, MAE-A and K30 were not taken into consideration as due to their low degradation temperature, the potential melting of crystalline trimethoprim could not be assessed via DSC. Still, the systems with these two polymers were analysed via DSC up to the degradation temperature and via XRPD.

Among polymers fully assessable by DSC, HPMCAS performed best: fully amorphous dispersions were obtained up to 30% (w/w) trimethoprim, and at 50% the system contained only a minor residual crystallinity of approximately 4% relative to the whole system. By contrast, HPMC, VA64 and EPO each exhibited residual crystallinity already at 10% drug load. For EPO this was expected based on the findings in melt quenching and its basic functional groups and the weakly basic API, where favourable interactions were not anticipated. The comparatively weak performance of VA64 and HPMC was more surprising because, as polymers with neutral functional groups, they typically stabilise weakly basic drugs more effectively than basic polymers. VA64 is frequently reported to form robust ASDs with weakly basic APIs by providing a hydrophobic microenvironment around the small molecules [53,175,176]. The present outcome therefore underscores the strength of trimethoprim's crystallisation driving force under melt processing and suggests that, for this class I rapid crystalliser, the neutral polymers' stabilising interactions were insufficient.

In XRPD analysis, all HPMCAS and HPMC samples were amorphous. For VA64, crystallinity was found only at 70% drug load (23% crystallinity, determined via DSC). With EPO, the 30% and 50% drug load showed a peak pattern suggesting crystallinity (13% and 41% residual crystallinity, respectively).

For K30 and MAE-A, the DSC analysis revealed no exothermic or endothermic signals up to 165 °C and 160 °C, respectively, at 10% and 30% drug load each. For K30, the 50% loaded sample showed a recrystallisation peak at 130 °C with an area equal to 12% crystallisation of the whole system, so that is the minimum amount that had to be amorphous after manufacturing. In the samples loaded with 10% and 30%, no such signals were observed. During XRPD analysis, all K30 samples were amorphous. For MAE-A, the 10% and 30% samples were XRPD-amorphous as well and did

not show any crystallisation or melting signals in the DSC up to 160 °C. The 50% samples were partly crystalline in XRPD analysis, and a melting endotherm was found in the DSC at 160 °C close to the end of the analysis cycle. For this reason, the signal could not be evaluated in regard to its area under the curve. A preceding crystallisation exotherm was not detected. Based on these findings, it cannot be stated whether the K30 samples were partially crystalline from the beginning, as XRPD can only detect residual crystallinity of at least 10% (see chapter 4.2.4). For MAE-A, 50% drug load could not be embedded as a homogeneous ASD via HME.

Ternary ASDs were manufactured with a polymer mixture of HPMCAS and VA64 in a 1:1 ratio and trimethoprim drug loads of 30% (w/w), 50% (w/w), and 70% (w/w). The 30% samples were completely amorphous, whilst in the higher loaded systems, residual crystallinity was found (50% drug load: 2.3%; 70% drug load: 20.1%, both relative to the whole system). The crystallinity was lower compared to binary systems with either HPMCAS (4.3% at 50% drug load) or VA64 (21.9% at 50% drug load, 22.8% at 70% drug load) alone, indicating a beneficial effect from the combination of the two polymers.

4.3.4 Spray Drying

The spray drying experiments started with a drug load of 30% (w/w). The residual crystallinity of the systems containing MAE-A and K30 could not be determined via DSC. As listed in Table 4.17, no homogeneous ASD could be manufactured with the other four polymers. With 30% drug load, crystallinity was detected within all samples. The crystallinity was lowest for EPO and VA64, in contrast to the findings from hot melt extrusion. For HPMCAS, the residual crystallinity was 13% in the 30% (w/w) loaded samples compared to a completely amorphous system in HME, and for HPMC it was 19% compared to 4% in HME. In literature, it is described that spray drying leads to a higher number of acid-base interactions between drug and polymer [177], but in the present work, no hints for stabilising interactions were found based on the residual crystallinity. Instead, spray-dried products were less stable compared to melt extruded ones. The determined drug content of spray-dried trimethoprim formulations is listed in Table 8.2 in the appendix.

A reason for the higher crystallinity could be the distinct thermal and kinetic profiles of the two processes, mainly the longer time the systems were exposed to increased temperatures in spray drying. In HME, the molten API-polymer blend exits the die at up to ~210 °C and is rapidly cooled, limiting the time window for molecular rearrangements and cluster formation in the softened matrix. In spray drying, however, particles accumulate and remain for 20-30 min at outlet temperatures of 60-70 °C. If drying is incomplete and residual methanol remains in the collected powder in the cyclone, nucleation and growth are favoured during this prolonged exposure.

Experiments also showed that disadvantages of a higher temperature setting can be compensated for if the higher temperature reduces residual solvents. This trade off was examined more directly at 50% drug load for HPMCAS and HPMC by using 90 °C instead of 80 °C inlet temperatures with

otherwise constant settings. Crystallinity was lower at 90 °C for both celluloses (11% versus 20% for HPMCAS and 18% versus 26% for HPMC). The cellulose polymers form a highly viscous mixture with methanol at higher concentrations, which slows down the drying process as it progresses [178]. An increase in drying temperature can accelerate the initial solvent evaporation, resulting in less residual solvent in the sample collection vessel and therefore lower molecular mobility and potential crystallisation, even though a higher product temperature is reached. Thus, optimisation requires balancing sufficient thermal driving force for complete drying with minimal thermal stress to the product.

As for HME, the samples containing MAE-A and K30 as matrix former could not be analysed. In XRPD-analysis, all samples with the two polymers were amorphous at drug loads of 30%, 50%, and 70% (w/w). In the thermal analysis via DSC, the 70% loaded MAE-A samples had a recrystallisation peak at 105 °C with an area corresponding to 19% crystallinity, meaning that at least 19 % of the whole system or, related to the 70% drug load, at least 27% of the API had to be amorphous initially. With K30, a recrystallisation signal was found in 50% loaded samples (112 °C) and in 70% loaded samples (94 °C). Here, the crystallised amount was 15% and 28% based on the whole system, respectively. The complete data on DSC- and XRPD-plots are listed in the appendix (Figure 8.17, Figure 8.18, Figure 8.19, and Figure 8.20).

4.3.5 Milling

Only selected systems were processed by ball milling of physical mixtures containing trimethoprim and the respective polymer. With HPMCAS, a 10% drug load was tested. Here, a residual crystallinity of 5% (relative to whole system) was found, meaning that half of the API could be transferred into the amorphous state. When reducing the drug load to only 5%, again half of the API remained crystalline, with no difference between milling times of 180 minutes and 360 minutes. For VA64, trimethoprim was almost completely crystalline for all tested drug loads. For MAE-A samples, no residual crystallinity could be measured in the DSC, but for all the samples, a recrystallisation event was observed at temperatures between 75 °C and 80 °C. For 15% drug load, the crystallisation corresponded to 3% of the whole system and for 20% drug load, it was a minimum of 4% amorphous drug with no difference between milling for 180 and 360 minutes. The 20% sample was stored at ambient conditions for three weeks with no changes in DSC results afterwards. The respective DSC plots are summarised in Figure 8.23 in the appendix.

As expected, the forces induced by milling were not sufficient to obtain systems with a reasonable amount of amorphous trimethoprim. The milling process lacks melting of the API and softening of the polymer as well as dissolving both components in a solvent. The mechanical input was not sufficient to embed the API into the polymer matrix. In literature, it was shown that hot melt extrusion and spray drying achieve more stable amorphous systems compared to milling techniques [179,180], in line with the findings in this work. As discussed before, trimethoprim is classified as class I

substance, meaning it is a rapid crystalliser. The method of milling might have a better effect for substances of class II or III with the advantage of minimum thermal stress and no inclusion of an organic solvent [181–183]. Still, for the pure drug substance, milling was the only operation that led to a partly amorphous product.

4.3.6 Residual crystallinity

A direct comparison of HME and spray drying across drug loads underscores the strong interplay between polymer chemistry, process thermal history, and solvent dynamics (see Figure 4.34). At 10% drug load, only HME was tested. HPMCAS yielded an amorphous system, whereas HPMC, VA64 and EPO showed comparable residual crystallinities. Interestingly, EPO did not perform worst; the mean residual crystallinity was even lower than for the neutral polymers, albeit with a higher standard deviation.

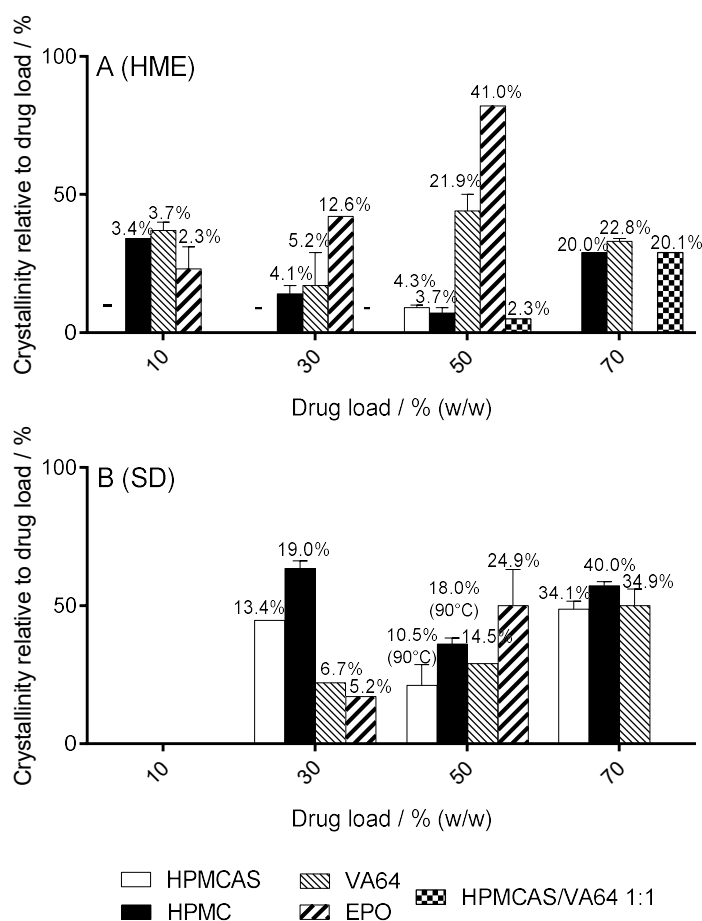


Figure 4.34 Residual trimethoprim crystallinity in the amorphous solid dispersions after preparation by hot melt extrusion (A) and spray drying (B). Given is the degree of crystallinity relative to the drug load calculated by the melting enthalpy. The values represent the mean $\pm$ standard deviation ($n = 3$). The residual crystallinity was zero for the melt-extruded systems with HPMCAS, 10% and 30% drug load. 70% drug load in EPO was not tested. In spray drying, no system was tested at 10% drug load. Ternary systems were only tested in HME (30%-70% drug load). The numbers on top of the bars give the crystallinity relative to the whole system (mean value of $n = 3$).

For 30% (w/w) drug load, the melt-extruded system with HPMCAS was still amorphous. In contrast, almost half of the API in the same system prepared by spray drying was crystalline. With HPMC, the crystallinity was also significantly lower after hot melt extrusion than after spray drying. VA64 performed similarly across the two methods, while EPO surprisingly performed better in spray drying than in HME. These observations are mostly consistent with the longer dwell times and residual solvent inherent to spray drying, which raise molecular mobility and hence crystallisation probability. When comparing the polymer types in spray drying, HPMCAS and HPMC performed worse than VA64 and EPO, as explained in section 4.3.4. The higher viscosity during the solvent evaporation step of the celluloses can promote a longer drying time and therefore more potential crystallisation. In the solvent-free process of hot melt extrusion, the polymers performed as expected with HPMCAS as the best, followed by HPMC and VA64. By eliminating solvent and providing rapid quenching, HME favours the development and “freezing in” of stabilising interactions.

At 50% drug load, HPMCAS could no longer form fully homogeneous ASDs by HME, yet its residual crystallinity remained lower than in spray drying. HPMC followed the same pattern. VA64 and EPO, by contrast, trended better in spray drying, albeit with large deviations among replicates. The performance of the polymer types was comparable for hot melt extrusion and spray drying with a tendency for the celluloses to perform best and EPO as the worst option. The assumption was made that the negative effects caused by the basic functional groups of EPO were more pronounced at higher drug loads. The other monomers, being butyl methacrylate and methyl methacrylate, can stabilise the amorphous trimethoprim with their hydrophobic moieties, but still, the basic structures of EPO are responsible for the occurrence of repulsion, which can result in the formation of clusters and subsequent crystallisation of trimethoprim. Opposed to cinnarizine, trimethoprim does not benefit to the same extent from the hydrophobic structures that could potentially compensate for the basic structures.

With lower drug loads, other effects might compensate for the lack of beneficial interactions, for example the mixing quality in extrusion and therefore the distribution of trimethoprim in the polymeric matrix. EPO has by far the lowest glass transition temperature of all tested polymers, even lower than the one of trimethoprim. Due to the early softening of the polymer and a longer time in the softened state during extrusion, the API could distribute over the whole system more evenly, hindering the formation of clusters. Polymers with a higher glass transition temperature might be able to form more molecular interactions with the API and thus stabilise the system, but a lack of even distribution could mean that these interactions cannot be fully developed. This limitation can partly be compensated for by increasing the extrusion temperature to enable the polymer to soften to a larger extent and to lower its melt viscosity. This enhances the mixing quality and promotes a more homogeneous API distribution in the system. However, higher temperatures increase the risk of thermal degradation of both polymer and API. In this study, extrusion temperatures were therefore maintained only slightly above the melting point of trimethoprim to balance these effects. In some

cases, even reaching the melting point was not possible due to polymer degradation constraints, highlighting the narrow processing window for achieving both sufficient mixing and thermal stability.

The ternary ASD (HME, 50% (w/w) drug load) possessed a lower residual crystallinity compared to the respective binary systems (4.7% compared to 8.5% and 43.8% for HPMCAS and VA64, respectively). The combination of both polymers increases the embedding potential and stabilises the amorphous state of the active ingredient. Trimethoprim has polar and non-polar structural moieties, leading to potential interaction sites with the acidic polymer HPMCAS as well as the more hydrophobic polymer VA64.

At 70% (w/w) drug load, EPO was not tested. HPMCAS was only tested in spray drying. HPMC and VA64, which were tested with both methods, both performed better in hot melt extrusion. Within one method of preparation, no differences were found in the results obtained by the single polymers. Even at the highest tested drug load, approximately half of the API could be transferred into the amorphous state in spray drying and even more in hot melt extrusion. Though crystals were present that could facilitate further crystal growth, all polymers could embed the amorphous API to a certain degree. Interestingly, the level of amorphous API increased with the drug load. As mentioned above, half of the 70% drug load were amorphous in the systems prepared by spray drying, meaning a total of 35% amorphous drug load for the systems. At 30% total drug load, not all of the API was amorphous, but a crystalline portion was found. This was approximately half of the API in certain cases, which supports the assumption that the polymers can embed a relatively high degree of amorphous API, but even small inhomogeneities or the presence of a crystal seed induce large-scale crystallisation. The crystallinity within the ternary ASD was 28.7%, which was lower than the one for the binary VA64 system (32.5%).

Finally, it was noted that the quantitative limits of amorphous incorporation established here depend on both the polymer's intrinsic stabilisation mechanisms and the process's capacity to limit molecular mobility during and immediately after phase change. HPMCAS consistently exhibited superior stabilisation of trimethoprim, especially under conditions that minimise residual solvent and maximise quench efficiency.

4.3.7 Glass transition temperatures

Besides the residual crystallinity, the glass transition temperature of the drug-polymer system can show differences between the manufacturing methods. Figure 4.35 shows how the T_g differs for the three manufacturing methods of melt quenching, hot melt extrusion and spray drying.

With HPMCAS, the samples from melt quenching showed a faster decrease of the mixed T_g with low drug loads and then an approximation to the T_g of the API. After hot melt extrusion, the value at 10% drug load (w/w) was lowered in comparison to that line, while the 30% and 50% (w/w) values

were a good fit. The spray-dried samples showed a stronger decrease of the T_g at 50% and 70% drug load (w/w). Residual methanol in the product can lower the system's glass transition temperature significantly due its softening effect.

With HPMC, the results for the T_g s in samples from hot melt extrusion and spray drying were identical, but below the results from melt quenching. Solvent effects can play a role, as also the extruded samples can take up water after manufacturing. In melt quenching, existing moisture was vaporised immediately before rapid cooling. Additionally, the increased mixing capacity in HME compared to melt quenching could induce a more homogeneous system which was able to form more molecular interactions. It was an interesting finding that the glass transition temperature had a minimum at 20% (w/w) drug load and then increased again to the one of trimethoprim. This behaviour had not been observed in previous studies.

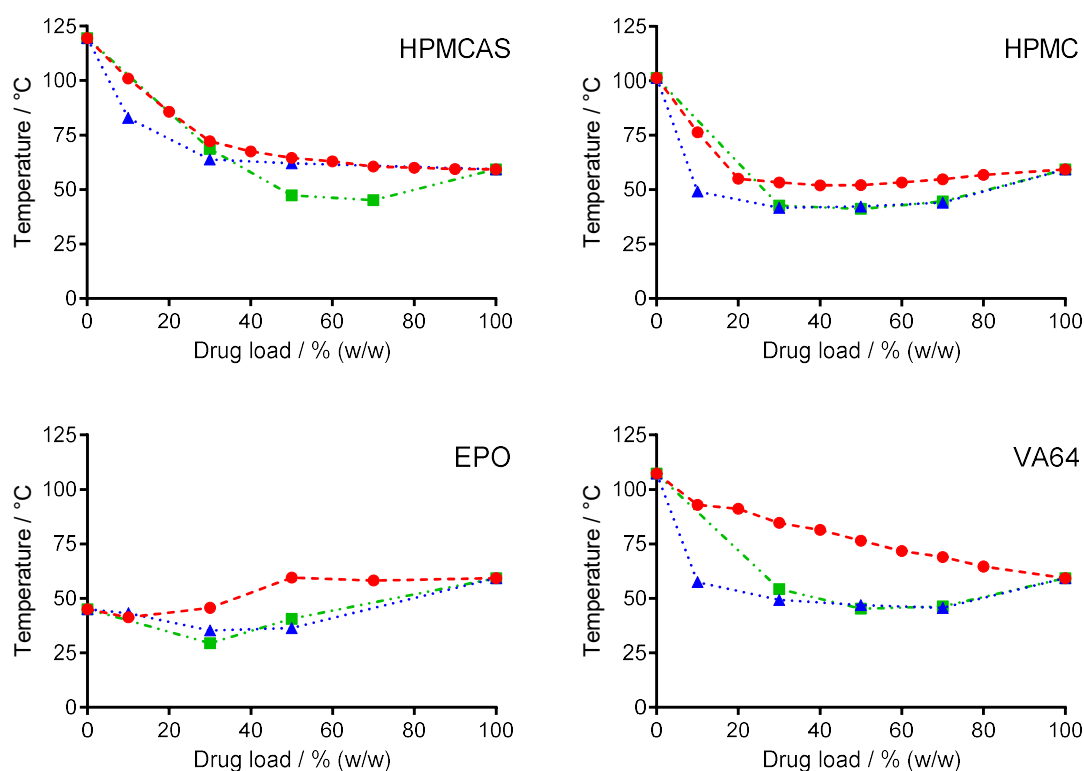


Figure 4.35 Glass transition temperatures of API-polymer-systems with HPMCAS, HPMC, EPO, and VA64. The temperatures are given for systems prepared by melt quenching (red circles), hot melt extrusion (blue triangles), and spray drying (green squares). Please note that there are no 10% drug load values for samples from spray drying.

For EPO and VA64, extruded and sprayed samples showed similar values, in both cases below the melt quenched samples. Especially VA64 has a high tendency to adsorb water (see chapter 4.3.9 and a review on polymeric carriers by Nair et al. [184]), which can influence the temperature of the glass transition as explained before. The second-heating T_g s were in line with the melt quenching results, proving that upon evaporation of humidity, the systems were equal.

The results show that there were no differences in the T_{gs} between hot melt extrusion and spray drying. Even though the crystallinity was different in most cases, which also means that the concentration of amorphous API was not equal, the measured T_{gs} overlapped. Different moisture or solvent levels could compensate for small differences in the concentration of amorphous API. Additionally, for high drug loads, the influence of the API content on the T_g gets smaller. In cases where the T_g of both API and polymer are close to each other, as it is with trimethoprim and EPO, the range in which the mixed T_g can result is limited.

4.3.8 Phase diagrams

Phase diagrams were developed for four different polymer-based systems to assess the solubility and stability limits of trimethoprim therein. These diagrams utilise the relationship between drug load and thermal transitions, specifically T_g and T_m , to identify the boundaries of stable system formation. The phase diagrams in Figure 4.36 show the measured values for the mixed glass transition temperature and the solubility temperature of trimethoprim in HPMCAS, HPMC, EPO, and VA64 together with the model curves of Gordon-Taylor and Flory-Huggins. For the Gordon Taylor model, the parameter k was fitted to the experimental results. Additionally, relevant storage temperatures for stability testing are displayed in orange (40 °C, 25 °C, 8 °C). A common problem for the calculation of the Flory-Huggins model based on melting point depression is the lack of values for low drug loads, as it is challenging to determine the melting point depression here [69].

Due to the high glass transition temperature of trimethoprim, all of the systems are in phase III at the shown storage conditions including room temperature. Phase IV, in which the glass transition temperature is exceeded and therefore, molecular mobility is facilitated to form clusters and crystals, cannot be reached due to the high glass transition temperature of trimethoprim. Still, also in phase III there is a certain degree of mobility and a potential for inhomogeneity over time, as the systems are only kinetically stabilised. The thermodynamically stable phase II can also not be reached at common storage conditions due to the course of the solubility line.

In conjunction with the findings from the ASD manufacture and analysis of the API, it was found that trimethoprim is challenging to embed into a polymeric carrier due to its propensity for rapid crystallisation upon cooling, when still in phase IV, or upon drying. The phase diagrams illustrate that if an ASD is successfully formed, even with high drug loads, the systems can be stabilised kinetically under standard storage conditions, but for this, the formed ASD needs to be fully amorphous in the beginning and homogeneous without phase separation.

It is crucial to acknowledge that the phase diagrams do not include the influence of humidity in the ASDs. Residual solvents from the manufacturing process or water uptake during storage can soften the formulation, meaning the T_g can be reduced to the selected storage temperatures. By this, the system could fall into phase IV where crystallisation is inevitable. This is especially troublesome

as climatic zones with increased humidity often employ accelerated temperatures as well, for example the ICH storage condition 40 °C with 75% relative humidity.

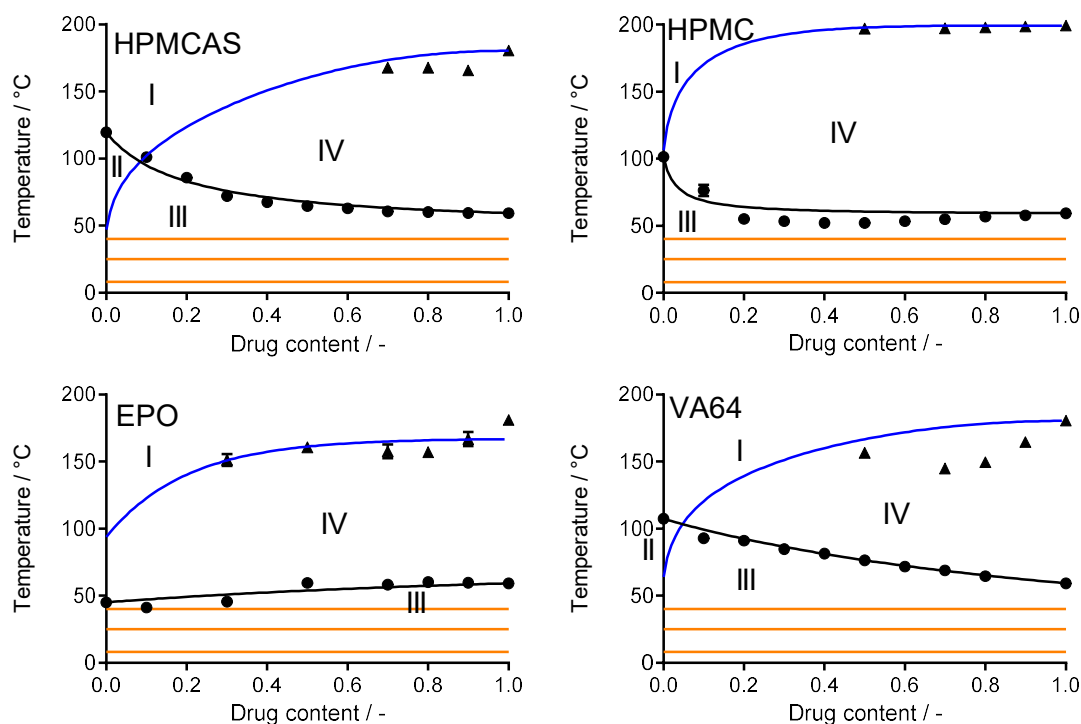


Figure 4.36 Phase diagrams of trimethoprim-HPMCAS, trimethoprim-HPMC, trimethoprim-EPO, and trimethoprim-VA64. Dots and triangles show the measured glass transition temperatures and solubility temperatures, respectively. The lines represent the calculated curves for Gordon-Taylor (black; fitted k), Flory-Huggins (dark blue) and different storage temperatures of 40 °C, 25 °C and 8 °C (orange). Roman numbers indicate the single phases.

4.3.9 Film casting and high-throughput screening

Whilst phase diagrams can mainly be used to estimate possible drug loads in ASDs prepared by melting techniques and the associated required process temperatures, two options for a solvent-based screening were tested for trimethoprim – film casting and a high-throughput ASD screening method.

In film casting, six polymers were tested at levels of 30%, 50%, and 70% drug load (w/w) and films were dried either in a LAF or in a vacuum drying cabinet. For EPO, HPMC, and K30, crystals were visible by microscopic analysis at 30% drug load with both drying methods (see Figure 4.37, left for K30). With VA64, crystals appeared at 50% drug load when dried in the LAF, but at 30% for the vacuum drying cabinet samples (Figure 4.37, right; more pictures for additional polymer-drug load-combinations with crystal detection are shown in the appendix in Table 8.5 and Table 8.6). HPMCAS samples were optically amorphous at 50% drug load, whilst crystals were visible at 70%, regardless of the drying method. For MAE-A samples, the outcome was dependent on the drying method: the 70% drug-loaded samples were amorphous when prepared in the LAF, but not after drying in the drying cabinet. A trend was therefore visible for more stable and amorphous systems

when casting and drying was performed in the LAF (see Table 4.18). Less particle contamination to start crystallisation was possible here. Additionally, the drying in the vacuum cabinet was combined with accelerated temperatures of 40 °C. Though the increased temperature and decreased air pressure should lead to a more rapid drying, which was thought to be beneficial for the stability of ASDs, the effect could be outperformed by both the higher molecular mobility by increased temperatures and the possibility of particle contamination while handling the glass screens outside of the LAF. As the samples were dried over night with both methods, the exact difference between the required drying times was not measured as well as the residual solvent content over time.

Table 4.18 Onset of visible crystallisation during film casting and high-throughput screening in % drug load (w/w). “-/-” means that the lowest tested drug load had been crystalline already.

Polymer	Film casting, vacuum dryer		Film casting, LAF		High-throughput screening	
	Highest amorphous load	Lowest crystalline load	Highest amorphous load	Lowest crystalline load	Highest amorphous load	Lowest crystalline load
MAE-A	50%	70%	70%	> 70%	60%	70%
EPO	-/-	30%	-/-	30%	10%	30%
HPMCAS	50%	70%	50%	70%	30%	70%
HPMC	-/-	30%	-/-	30%	20%	30%
VA64	-/-	30%	30%	50%	20%	50%
K30	-/-	30%	-/-	30%	20%	30%

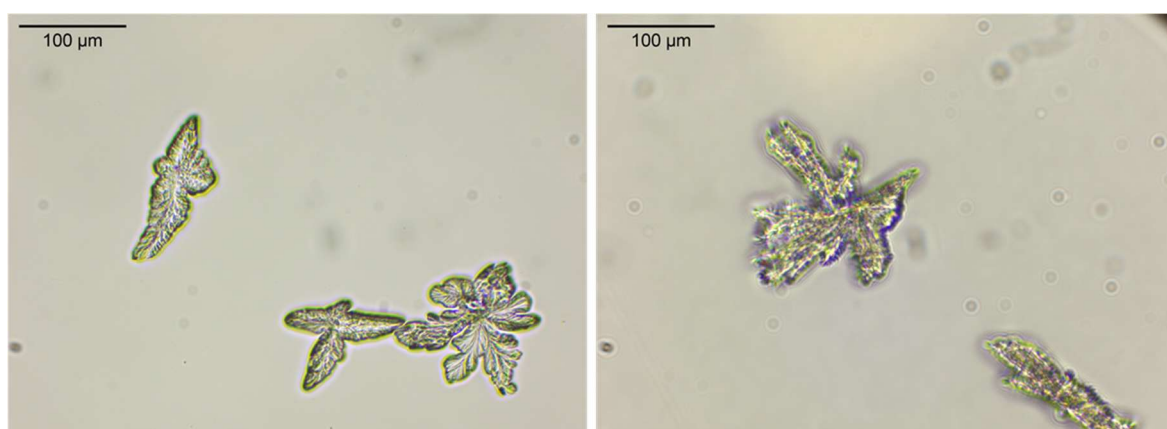


Figure 4.37 Crystals formed during film casting. Left: K30 with 30% trimethoprim load prepared in the laminar airflow box. Right: VA64 with 30% trimethoprim load prepared in the vacuum drying cabinet.

A high-throughput screening was performed in 48-well plates to get a more accurate determination of the drug load. Here, an earlier onset of crystallisation compared to the film casting was found. The highest drug loads without visible crystallisation were 10% for EPO, 20% for HPMC, K30 and VA64, 30% for HPMCAS and 60% for MAE-A. It is assumed that the drying time was higher for the well plates compared to the glass screens, the latter having a layer thickness of only 100 µm. Based on a given area of 0.95 cm² and a volume of 200 µL per well, the fill level can be calculated to be 2.1 mm, which is a more than twentyfold increase compared to the glass plates.

Not only had the drug load that initiated the crystallisation process differed between the polymers, but also the shape of the crystals was subject to substantial variation, as can be seen in Figure 4.38. For K30 and VA64, crystals were formed from elongated, slender strands in intricate patterns, while with other matrix forming polymers, they manifested as rounded structures, for example for HPMC. It is known from literature that there are at least four polymorphs of trimethoprim, varying in the shape of their crystals [171]. The pictures taken from the crystals in the polymer mixtures cannot be attributed to the distinct forms, as their appearance depends on specific interactions between the drug and functional groups of the polymers as well as the polymers' glass transition temperatures. Additionally, a difference in nucleation type can influence the crystal shape, being either homogeneous (spontaneous) or heterogeneous (due to interfaces or defects).

A scale-up in the vacuum drying cabinet based on the highest drug load without crystallisation from high-throughput screening was performed (MAE-A with 60% drug load, EPO with 10% drug load, HPMCAS with 30% drug load and HPMC, VA64 and K30 with 20% drug load each). For this purpose, solutions in methanol were prepared with a solid content of 1.2% (w/V), consisting of trimethoprim and the respective polymer. After drying in the vacuum drying cabinet in silicone wells and subsequent milling, the XRPD analysis revealed that all samples were crystalline except for the one with 30% drug load in HPMCAS. The discrepancies between high-throughput screening and the larger scale experiment could be explained by the differences in the layer thickness and the variations in drying time. This assumption was supported by an additional experiment with MAE-A with 60% drug load, where the solution was not distributed over four, but over twelve silicone wells, reducing the height by a factor of three. Here, the sample was amorphous.

The crystalline samples and the amorphous one with 30% drug in HPMCAS were stressed at 40 °C and 75% relative humidity in the DVS. The temperature was constant, but the humidity was increased with a ramp before being constant and then decreased again. During the increase, all samples showed a raise in the mass change due to water uptake, most pronounced for K30 and VA64. When reducing the relative humidity in the chamber, the water was desorbed and the samples gained their original mass at zero percent humidity. During the constant phase at 75% relative humidity, it was expected that the samples were in an equilibrium with their surrounding and no water uptake or release takes place. As Figure 4.39 (left) shows, the amorphous material with HPMCAS showed a decrease in mass during this phase. A material in an amorphous state can absorb humidity to a larger extent than a crystalline one of the same chemical composition. In the process of crystallisation of amorphous materials, the exclusion of water is attributable to the closure of gaps in the material by tighter and stronger bonds in the crystal [185,186]. When releasing water, the mass of the sample is reduced. This hypothesis is confirmed by subsequent XRPD analysis as shown in Figure 4.39 (right). Peaks indicating crystalline domains appear in the sample after stress-testing in the DVS. The former amorphous system with HPMCAS crystallised during the stress-test at increased relative humidity and temperature, indicating that the amorphous sample was not stable at these conditions.

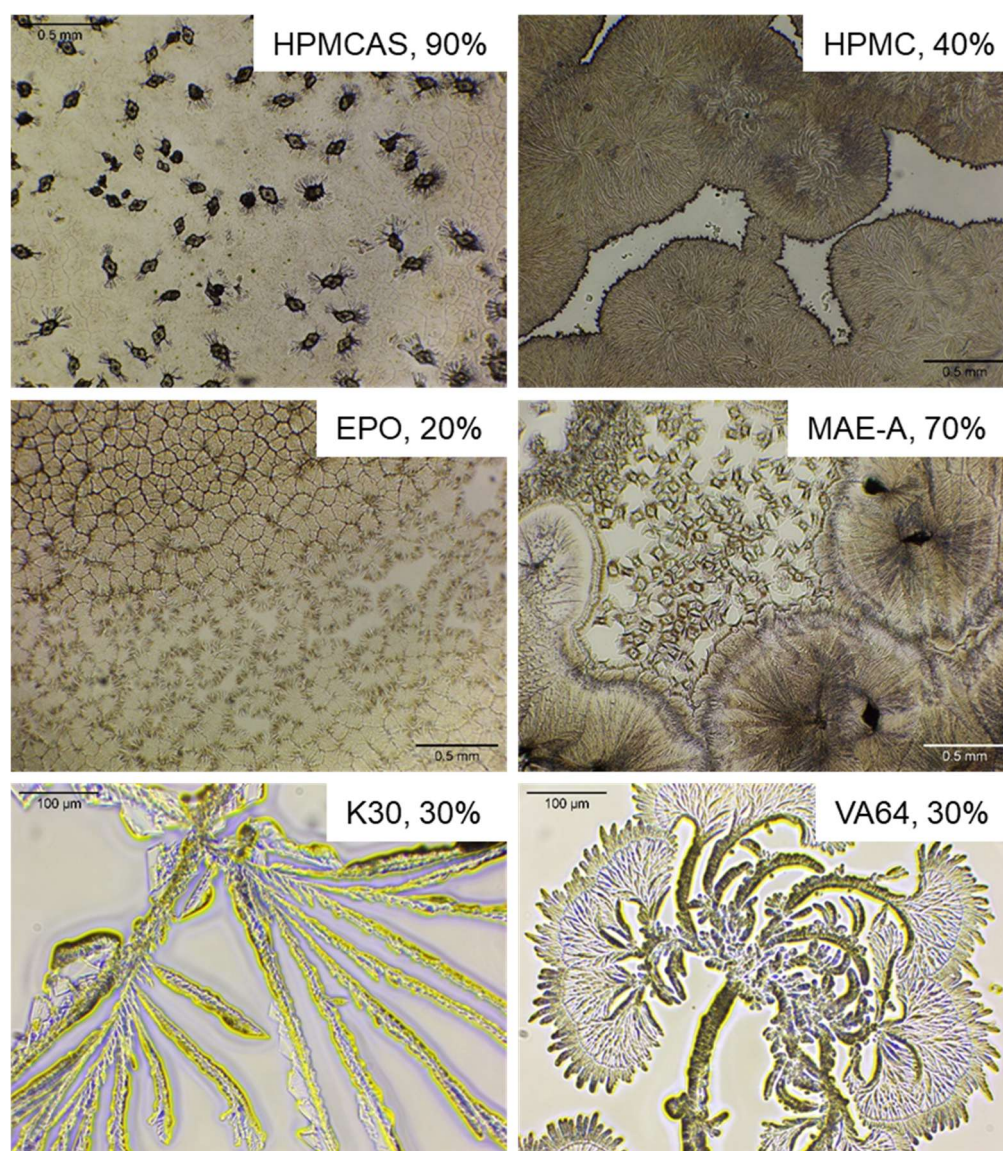


Figure 4.38 Crystals formed in the well plates during high throughput screening. Given is the polymer type together with the drug load of trimethoprim.

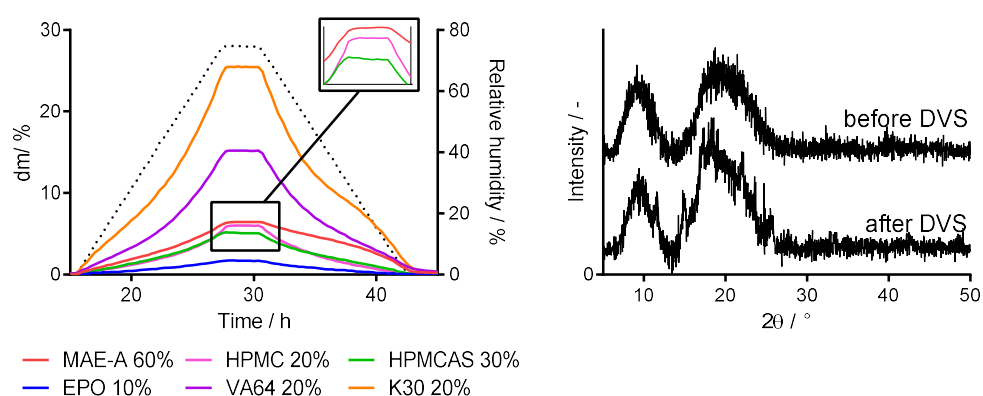


Figure 4.39 Amorphous solid dispersions stressed via dynamic vapour sorption. Left: Change in mass over time of the amorphous solid dispersions prepared by solvent evaporation at 40 °C and 75 % relative humidity. The dashed line shows the relative humidity; the solid lines show the mass change in percent based on the lowest net weight; the legend shows the allocation of the polymers together with the drug load (w/w). Right: XRPD of HPMCAS ASDs before and after stress testing in the DVS.

4.3.10 Solubility enhancement

The thermodynamic solubility of trimethoprim was assessed in various aqueous and organic solvents (Table 4.19). With an aqueous solubility of 0.5 mg/mL, trimethoprim is classified as very slightly soluble according to the European and the United States Pharmacopoeia. Though the solubility is significantly higher than the one of cinnarizine, it still represents a challenge for aqueous drug dissolution. The solubility is partly pH-dependent, but the effect is not as pronounced as for cinnarizine. Also, the addition of bile salts and lecithin in FaSSIF pH 6.8 compared to phosphate buffer pH 6.8 did not increase the saturation solubility. In organic solvents, the solubility depends on the solvent's polarity to a large extent: with a poor solubility in octanol, trimethoprim is slightly soluble in methanol and ethanol.

The influence of polymeric additions on the solubility in phosphate buffer pH 6.8 was tested as listed in Table 4.20. With all polymers, the solubility increase was smaller than tenfold. For 5% addition of EPO and Soluplus, the solubility was even reduced compared to buffer. For the other polymers, a concentration dependent effect was observed, mostly with only small differences. The highest increase was induced by 5% PSSA addition, for which also the pH shifted to the largest extent. For MAE-A, K30, PAA, and PSSA, there was at least a doubling of solubility; these polymers except for K30 have acidic structural moieties. Due to the small and mostly not significant effects, no ranking of the advantageous effects was performed.

Table 4.19 Saturation solubility (thermodynamic) of trimethoprim in various organic and aqueous solvents (mean $\pm$ standard deviation, $n = 3$). For organic solvents, the solubility is given in mg/mL; for aqueous solvents, the values are given in $\mu\text{g/mL}$.

Solvent	Solubility [mg/mL]	Solvent	Solubility [$\mu\text{g/mL}$]
Methanol	9.4 ± 0.5	Water	500 ± 67
Ethanol	5.5 ± 0.2	Phosphate buffer pH 6.8	1670 ± 38
Octanol	0.6 ± 0.0	FaSSIF pH 6.8	1070 ± 115
		1 mM HCl	720 ± 1
		10 mM HCl	3710 ± 110
		0.1 M HCl	2840 ± 110

Table 4.20 Changes in trimethoprim thermodynamic solubility following polymer addition to phosphate buffer pH 6.8 ($n = 3$). Given is the factor of concentration increase and the pH measured while sampling.

Polymer	Solubility change factor		pH		Colour-code	
	1%	5%	1%	5%	Factor	pH-shift
MAE-A	2.8	3.3	6.64	5.93	1-9	$+ > 0.10$
EPO	1.0	0.8	7.23	7.63	10-99	$+ 0.06-0.10$
HPMCAS	1.2	1.4	6.95	6.51	100-999	± 0.05
HPMC	1.1	1.3	6.91	6.99	1000-9999	$- 0.06-0.10$
VA64	1.2	1.5	7.05	7.02		$- 0.11-1.0$
K30	1.5	2.8	6.90	6.87		$- 1.0-2.0$
PAA	2.3	3.7	6.18	4.04		
PSSA	3.6	5.6	6.14	1.56		
Soluplus	1.3	0.8	6.82	6.83		
PEG 6,000	1.2	1.4	6.85	6.87		

4.3.11 Partition coefficient

In addition to solubility testing, the partition coefficient in aqueous buffer or acidic solutions and octanol was determined. A minor decrease of trimethoprim concentration in the organic octanol phase was found when the aqueous medium was changed from phosphate buffer pH 6.8 to 0.1 M HCl, meaning that more API diffused into the aqueous medium. This fits to the slightly increased solubility of trimethoprim at lower pH values.

The addition of polymers caused an increase in the partition coefficient (Figure 4.40). In all instances, more drug was partitioned into the octanol phase versus the aqueous phase, which was not expected due to the lower thermodynamic solubility of trimethoprim in octanol compared to phosphate buffer. The observed increase in the partition coefficient upon polymer addition indicates a preferential partitioning of the drug into the organic phase, suggesting that polymers may exert a negative effect on the transfer into the aqueous phase. The polymer concentration was 1% in all cases, which makes an increased viscosity unlikely to be the cause for the reduced diffusion.

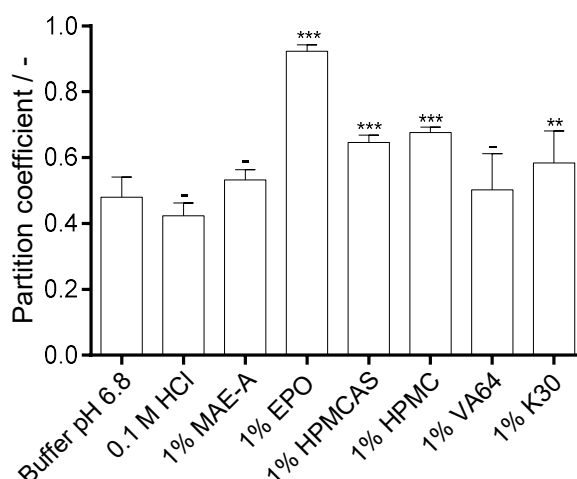


Figure 4.40 Partition coefficient of trimethoprim depending on the polymer addition to the aqueous phase. The organic phase was octanol in all cases; when polymers were added to the buffer in the aqueous phase, the concentration was 1% (w/w). The displayed values represent the mean $\pm$ standard deviation ($n = 3$). *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$, all referring to the comparison with phosphate buffer without polymer addition.

Among the tested polymers, the partition coefficient was highest with EPO in the aqueous phase, meaning that with EPO, the least amount of trimethoprim was dissolved in the aqueous phase. This is consistent with the findings from solubility testing, where the addition of EPO did not lead to an increase in trimethoprim's solubility. The solubility increase was highest with MAE-A, which fits to the low partition coefficient with this polymer. However, the overall results do not align fully with expectations based solely on the outcomes from solubility enhancement tests with the polymers, indicating a more complex interplay between polymer-drug interactions and phase partitioning. Additionally, the partitioning of polymer molecules into the organic phase or a localisation at the interface could bias the outcomes of the testing.

4.3.12 Dissolution testing – USP Apparatus II

The dissolution testing in USP Apparatus II was performed with milled extrudates. As HPMC ASDs were too soft to be milled at 10% and 50% drug load with both the planetary ball mill and the cryomill, the HPMC samples were not tested.

For the 10% ASDs, dissolution behaviour was examined at two total masses (200 mg and 2000 mg) corresponding to 20 mg and 200 mg of trimethoprim, respectively (see Figure 4.41, A and B). At a total mass of 200 mg, the ASDs produced with VA64 and K30 dissolved rapidly, reaching complete release within ten minutes. In contrast, samples with MAE-A and EPO required approximately 120 minutes to approach comparable levels. HPMCAS-based ASDs did not achieve complete dissolution. The crystalline API (20 mg), included for comparison, dissolved to less than 50% of the theoretical maximum (100 $\mu\text{g/mL}$), even though this value lies well below the saturation solubility of 1070 $\mu\text{g/mL}$. This indicates slow release kinetics for trimethoprim.

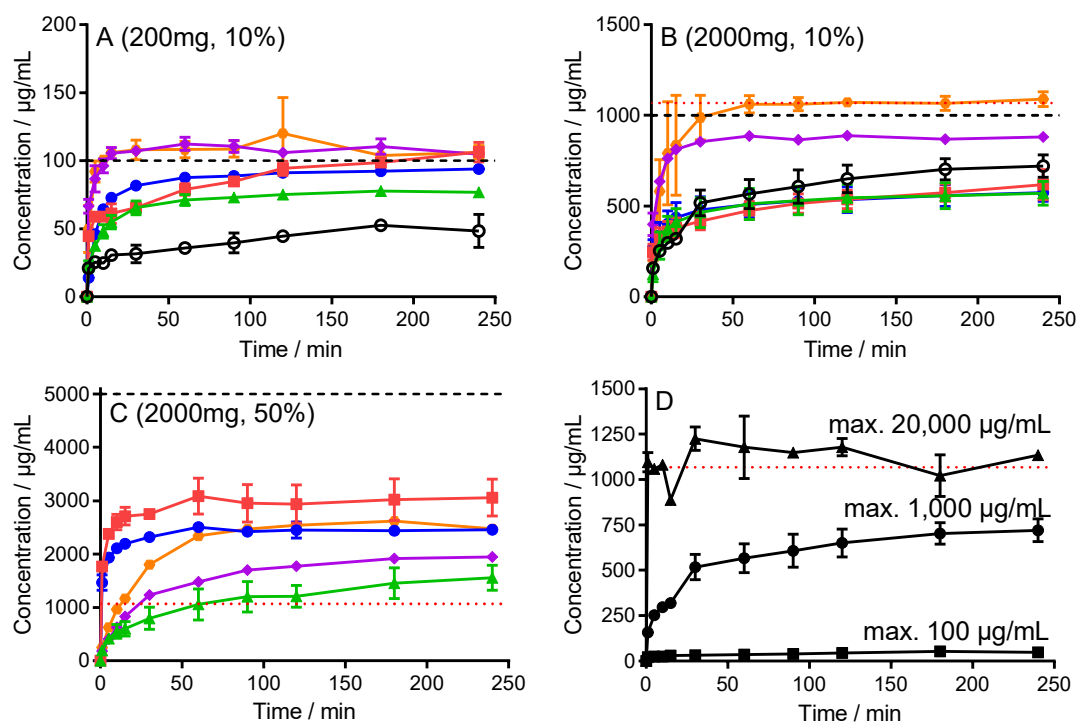


Figure 4.41 Dissolution profiles of trimethoprim ASDs prepared by hot melt extrusion in FaSSIF medium using USP Apparatus II. A – C: ASDs with the polymers MAE-A (red squares), HPMCAS (green triangles), EPO (blue circles), VA64 (purple rhombuses) and K30 (orange hexagons). Black empty circles represent crystalline trimethoprim at the same mass as in the ASDs. The dashed black line represents a drug release of 100%; the dotted red line indicates the thermodynamic solubility of trimethoprim in FaSSIF. D: Crystalline trimethoprim at different amounts of 20 mg (squares), 200 mg (circles) and 4000 mg (triangles). All values are shown as mean $\pm$ standard deviation of $n = 3$.

The rapid dissolution observed for K30 and VA64 samples aligns with previous findings on their dissolution-enhancing properties [36,159,187,188] and the observations in chapter 4.2.8 for cinnarizine. Trimethoprim embedded in HPMCAS exhibited the slowest dissolution.

The better performance of MAE-A and especially EPO was unexpected. EPO is not soluble at the testing pH and was expected to enclose the API. After the experiment, visible particles were observed in the medium which had not dissolved. Trimethoprim was released from the particles without complete particle dissolution. The reduced particle size by milling minimised the diffusion distance required for API molecules to reach and dissolve into the surrounding medium, enabling dissolution without complete dissolution of the polymer matrix. The disintegration of the systems was sufficient to enable drug release.

At the higher test mass of 200 mg API, absolute dissolution values increased but still only reached about 70% of the maximum expected value. Among the ASDs tested at 2000 mg total mass, K30 and VA64 again showed the best performance. K30 reached complete dissolution after 60 minutes, while VA64 approached a plateau of around 90% after 30 minutes. HPMCAS, MAE-A, and EPO initially performed slightly better than the API but ultimately fell below it, reaching only about 60-70% drug release. The superior dissolution behaviour of K30 and VA64 is attributable to their rapid polymer dissolution. The cause of the observed loss in VA64 performance remains unclear, but could be linked to inhomogeneities in the drug content. The inability of the other polymers to surpass pure API release suggests the possibility of polymer related saturation effects: since 100% dissolution would have remained below the saturation solubility, achieving complete dissolution should have been theoretically feasible for all formulations.

Solid-state analysis via XRPD and DSC provided further insights. ASDs with K30 and MAE-A were amorphous in XRPD, and the sample with HPMCAS was amorphous in both XRPD and DSC even though exhibiting the worst dissolution behaviour. VA64 and EPO samples displayed partial crystallinity (4% and 2%, respectively), but still VA64 systems performed unexpectedly well in dissolution testing. The degree of residual crystallinity did not serve as a predictive measure for either the quantity or the rate of drug release.

When the 50% drug loaded ASDs were tested at a total mass of 2000 mg, a 100% release would have corresponded to a fivefold supersaturation (Figure 4.41, C). All tested formulations achieved supersaturation to some degree, although none reached full dissolution. MAE-A exhibited the strongest performance, reaching 60% release after 60 minutes and maintaining a stable concentration at roughly three times the saturation solubility without precipitation. This behaviour indicates favourable interactions between the polymer and the API, stabilising the dissolved state. EPO followed a similar profile but reached only about 50% of maximum release. HPMCAS again showed the weakest performance, achieving only 33% dissolution and displaying no clear plateau, suggesting that dissolution might continue slowly over time. For HPMCAS, it is known that though the initial

dissolution is slower compared to other polymeric systems, it is capable of stabilising the supersaturated state of a dissolved API for multiple hours [26,61]. K30 outperformed VA64 at this drug load, plateauing at around 45% after 90 minutes, whereas VA64 levelled off at approximately 40% after 180 minutes.

The influence of polymer dissolution speed decreased as the polymer/API ratio was reduced, explaining the reduced effectiveness of VA64 and K30 samples. Solid-state characterisation revealed that HPMCAS exhibited the lowest residual crystallinity among the 50% ASDs, despite its poor dissolution performance. The gel-formation of celluloses upon swelling in aqueous media can explain the reduced drug release, as the molecules need to diffuse through the highly viscous layer around the dissolving particles. VA64 and EPO, which performed better, showed higher residual crystallinity. For K30 and MAE-A, the crystallinity could not be determined; however, K30 appeared amorphous in XRPD, while MAE-A exhibited partial crystallinity.

Across all dissolution profiles of the 10% samples, plateaus were reached or approached, with no significant concentration drops, which is consistent with the fact that all values remained below saturation solubility.

The dissolution behaviour of crystalline trimethoprim at 20 mg and 200 mg (directly comparable to the 10% ASD doses) followed the same trend: although concentrations rose steadily over time, full dissolution was never achieved, even under apparent sink conditions. An additional test using 4 g was conducted to assess the behaviour at higher concentrations (Figure 4.41, D). At this level, 20 mg/mL would correspond to 100% release, whereas the saturation solubility was determined to be 1070 µg/mL. The values rapidly reached the saturation solubility within one minute and subsequently fluctuated around this level until the end of the dissolution study.

The slow dissolution kinetics of trimethoprim suggest that equilibration requires more than four hours. Literature describes a difference between a saturation “within” and “outside” the dosage form, meaning in the dissolution medium [189]. Drug dissolution can be hindered by an inner saturation of the dosage form, even if sink conditions are given in the medium. This could provide an explanation for the slow dissolution observed in ASDs, where the dissolution speed of the polymer itself influences the overall process together with the slow dissolution of the API. However, given that trimethoprim was added to the medium without the presence of a carrier or matrix former, it is not possible to apply an “inner” saturation. In addition, the medium was stirred to ensure an even distribution of undissolved particles. This distribution was checked visually, and it was ascertained that no clusters or lumps had formed and that no particles were floating.

For a better comparison of the total released amount of trimethoprim that would have been available for permeation processes, the area under the curve was calculated for the dissolution plots from $t = 0$ to $t = 240$ minutes and is depicted in Figure 4.42. Included are the results for crystalline trimethoprim at two mass levels, equivalent to the trimethoprim amount in 10% loaded ASDs with

200 mg and 2 g sample amount. The drug release is higher for 50% loaded samples, as expected due to the higher total available mass. Still, the only cases where the AUC increases by the factor of approximately five are with MAE-A and EPO. For HPMCAS, VA64 and K30, the AUC of the 50%-loaded samples is twice as high as the one of the 10%-loaded samples, showing that the drug release potential is lower compared to that of 10% loaded samples.

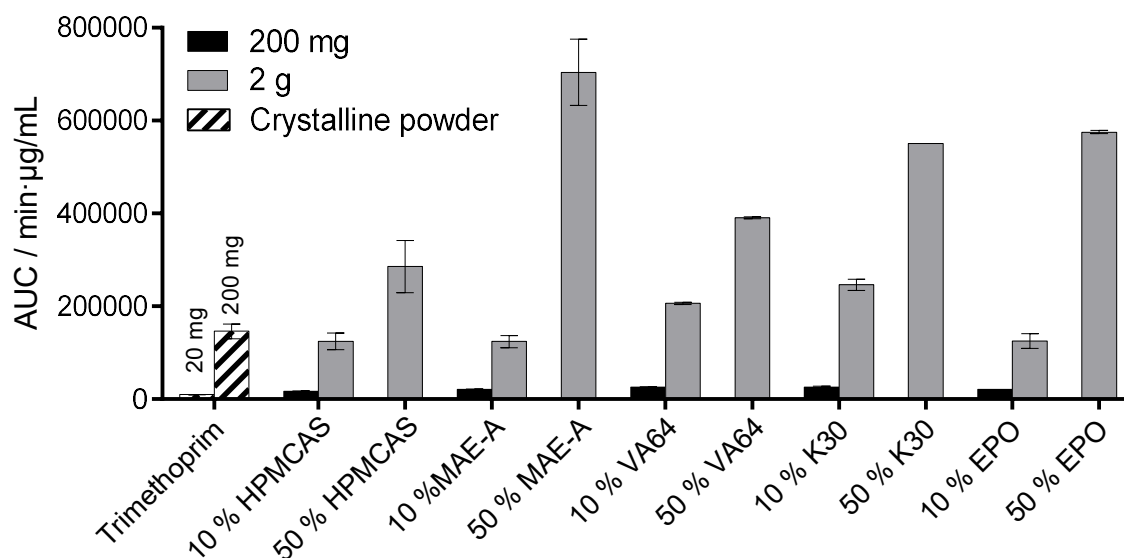


Figure 4.42 AUC of dissolution profiles of trimethoprim ASDs from $t = 0$ min to 240 min. The results are shown for 200 mg milled extrudates (black columns), 2 g milled extrudates (grey columns), and crystalline powder at two mass levels (hatched columns). Displayed are the mean $\pm$ standard deviation ($n = 3$).

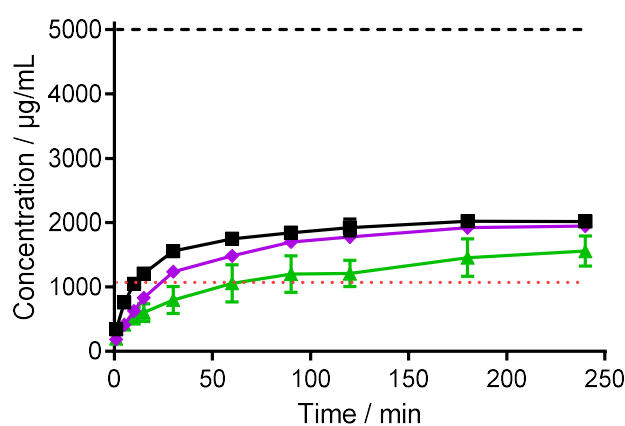


Figure 4.43 Dissolution profile of trimethoprim ternary ASD prepared by hot melt extrusion in FaSSiF using USP Apparatus II (50% trimethoprim – 25% HPMCAS – 25% VA64; black circles). The dashed black line represents a drug release of 100% (2000 mg of 50% (w/w) ASD), the red dotted line indicates the thermodynamic solubility of trimethoprim. The respective binary systems are added in green (50% TMP + 50% HPMCAS) and purple (50% TMP + 50% VA64). All values are shown as mean $\pm$ standard deviation of $n = 3$.

For the ternary ASD prepared by HME with HPMCAS and VA64, the dissolution profile showed drug concentrations above the thermodynamic solubility, but no complete drug dissolution was

achieved (Figure 4.43). The overall release rate was higher than that of HPMCAS binary systems and comparable to VA64 binary systems, both with 50% drug load, with a slightly faster release observed in the initial phase. The ternary system did not provide a clear or significant improvement in dissolution behaviour. Still, analyses on the residual crystallinity indicated a higher solid-state stability, suggesting a potential advantage of the ternary formulation from a stability perspective rather than from a dissolution perspective.

4.3.13 Dissolution testing – TFAM

To better simulate drug absorption and assess not only dissolution but also membrane permeation, additional studies were conducted using the TFAM setup. Here, the polymers could not pass the membrane due to their molecular size, but API was constantly removed from the system by membrane diffusion. The samples obtained by spray drying were examined in regards to their dissolution kinetics in the TFAM. ASDs with all polymers were investigated except for EPO. The saturation solubility of trimethoprim in the dissolution medium (30 mM phosphate buffer pH 6.8) was determined to be 864 µg/mL. The mass of the tested units was adapted to a quantity at which a 100% drug release would exceed the saturation solubility by a factor of ten. The mass of the API remained constant across the formulations, necessitating greater total sample mass for formulations with lower drug load.

Spray-dried ASDs with HPMCAS demonstrated the highest supersaturation for the API among the tested polymers but did not maintain the concentration until the end of the dissolution period (Figure 4.44-A). Samples with 30% drug load reached a higher maximum concentration than those with 50% drug load. This finding is in line with the so-called “falling-of-the-cliff” effect, which describes the fact that the dissolution rate of an API in an amorphous polymeric system drastically decreases above a certain drug load, until which the API and the polymer dissolve in a congruent manner and above which the dissolution is no longer polymer-governed [18,36,159]. After four hours, HPMCAS samples fell below saturation solubility, most likely due to crystallisation facilitated by seed formation. The maximum concentrations, which reached 2000-3000 µg/mL, exceeded those obtained in USP Apparatus II, where no concentration drop occurred. HPMC-based ASDs generated only slight supersaturation but maintained stable concentrations throughout, reflecting a more controlled dissolution pattern. Residual crystallinity in the HPMCAS ASDs was 13% at 30% load and 11% at 50% load; for HPMC at 30% load, residual crystallinity was 19%.

VA64 and K30 spray-dried ASDs showed similar dissolution behaviour, with slightly higher concentrations than with HPMC at the beginning but comparable profiles over the course of dissolution (Figure 4.44-B). With K30, the API concentration fell below saturation solubility towards the end of the experiment. 30%-loaded VA64 ASDs had a residual crystallinity of 7%. MAE-A showed drug load dependent behaviour. At 30%, dissolution progressed slowly but ultimately reached the saturation solubility. At 70%, a brief supersaturated state developed between 30 and

60 minutes, followed by precipitation, resulting in concentrations below saturation solubility (Figure 4.44-C). Differences in the distribution of dissolved drug between the dissolution compartment and the permeate were observed for MAE-A. At 30% drug load, the concentration in the dissolution compartment was substantially higher than in the permeate, whereas at 70% drug load, both values were more closely aligned. After 90 minutes, the dissolution compartment / permeate concentrations were 1390 $\mu\text{g/mL}$ / 730 $\mu\text{g/mL}$ for the 30% ASDs and 1440 $\mu\text{g/mL}$ / 1240 $\mu\text{g/mL}$ for the 70% ASDs. This pattern did not occur with other polymers. One possible explanation is that polymer-API interactions hindered trimethoprim permeation across the membrane. Because the membrane's molecular weight cut off of 5 kDa excludes both the polymer and any API fractions bound to or interacting with them, a larger polymer fraction, as in the 30% formulation, would naturally restrict permeation more strongly.

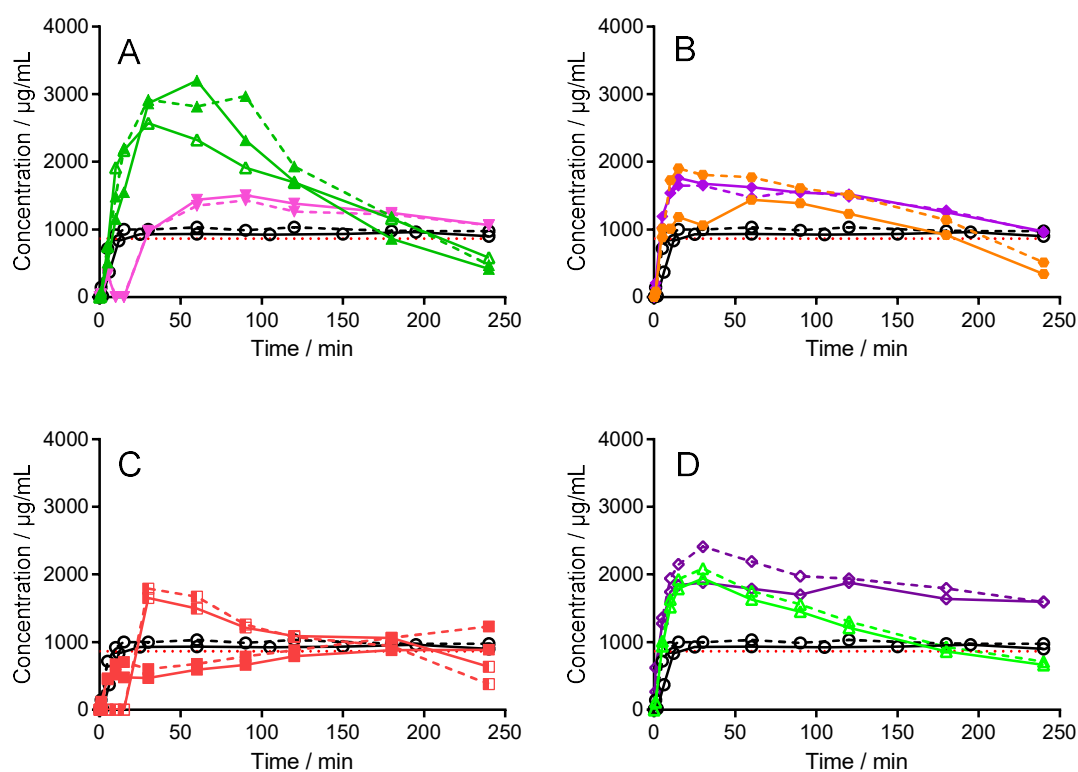


Figure 4.44 TFAM dissolution profiles of trimethoprim ASDs in phosphate buffer. Double determinations are indicated with solid and dashed lines. Black empty circles show crystalline trimethoprim. The dotted line represents the thermodynamic solubility. A – C: ASDs prepared by spray drying with HPMCAS (green), HPMC (pink), VA64 (purple), K30 (orange) and MAE-A (red). D: ASDs prepared by hot melt extrusion with HPMCAS (light green) and VA64 (dark purple). Drug loads are 30% (filled symbols), 50% (empty symbols), and 70% (half-filled symbols; only for MAE-A).

When HME formulations were tested in the TFAM setup, the 50% HPMCAS ASD reached lower maximum concentrations than its spray dried counterpart and remained slightly below it throughout most of the experimental period (residual crystallinity 4% for HME versus 11% for spray drying). By the final sampling point, the two methods converged (Figure 4.44-D). VA64 milled extrudates at

50% drug load (22% residual crystallinity) showed better dissolution behaviour than the spray-dried 30% VA64 formulation and exhibited a less pronounced decline. Despite the lower polymer content compared to the 30% spray dried samples, stabilisation of the supersaturated state was more effective.

AUC analysis revealed that from the 30% MAE-A formulation, less trimethoprim was released compared to the crystalline API (see Table 4.21). Among the tested formulations, 30% HPMCAS (SD) and 50% VA64 (HME) demonstrated the highest overall performance, indicating their superior dissolution properties. VA64 was the second-best polymer to enhance trimethoprim dissolution at 30% drug load considering the spray drying experiments. Comparison of the 50% HPMCAS formulations produced by spray drying and HME revealed a larger total AUC for the spray-dried samples. Despite its higher residual crystallinity, the spray-dried ASD reached supersaturation more rapidly, compensating for its sharper decline due to crystallisation and the lower final concentration reached after four hours.

Table 4.21 AUCs for the TFAM experiments. Given are the mean values of $n = 2$ in the unit of min·mg/mL.

Drug load [% (w/w)]	MAE-A	HPMCAS	HPMC	VA64	K30
30 (spray drying)	191.8	420.0	274.9	333.4	288.3
50 (spray drying)		383.3			
50 (hot melt extrusion)		303.3		436.1	
70 (spray drying)	247.3				
Crystalline trimethoprim: 225.9					

4.3.14 ¹H-NMR analysis

¹H-NMR titration series were performed with trimethoprim and certain polymers, both in methanol-d₄ and in DMSO-d₆. In DMSO-d₆, it is possible to detect signals of the protons bound in the primary amines (see Figure 4.45).

Only protons on the positions E, F and G are aliphatic ones, therefore found in the upfield region of the spectrum. The aromatic positions A and B are the two most downfield signals. The primary amines lead to relatively weak signals compared to the aromatic ones, though more protons are present. In the aprotic solvent DMSO-d₆, there is residual water that can enable a proton exchange and therefore weaken the signals.

The observed chemical shift patterns reveal polymer-drug interactions that vary with solvent and protonation environment (Figure 4.46). For position A, an upfield shift is consistently induced by polymer addition. Interestingly, when testing MAE-A in methanol-d₄, not only proton position A is affected by an upfield shift, but all the proton positions. A shift to the high field means that the protons are more shielded by a higher electron density. Due to the aromatic system and the two attached primary amines, the electron density in the ring is high as a cause of the inductive effect.

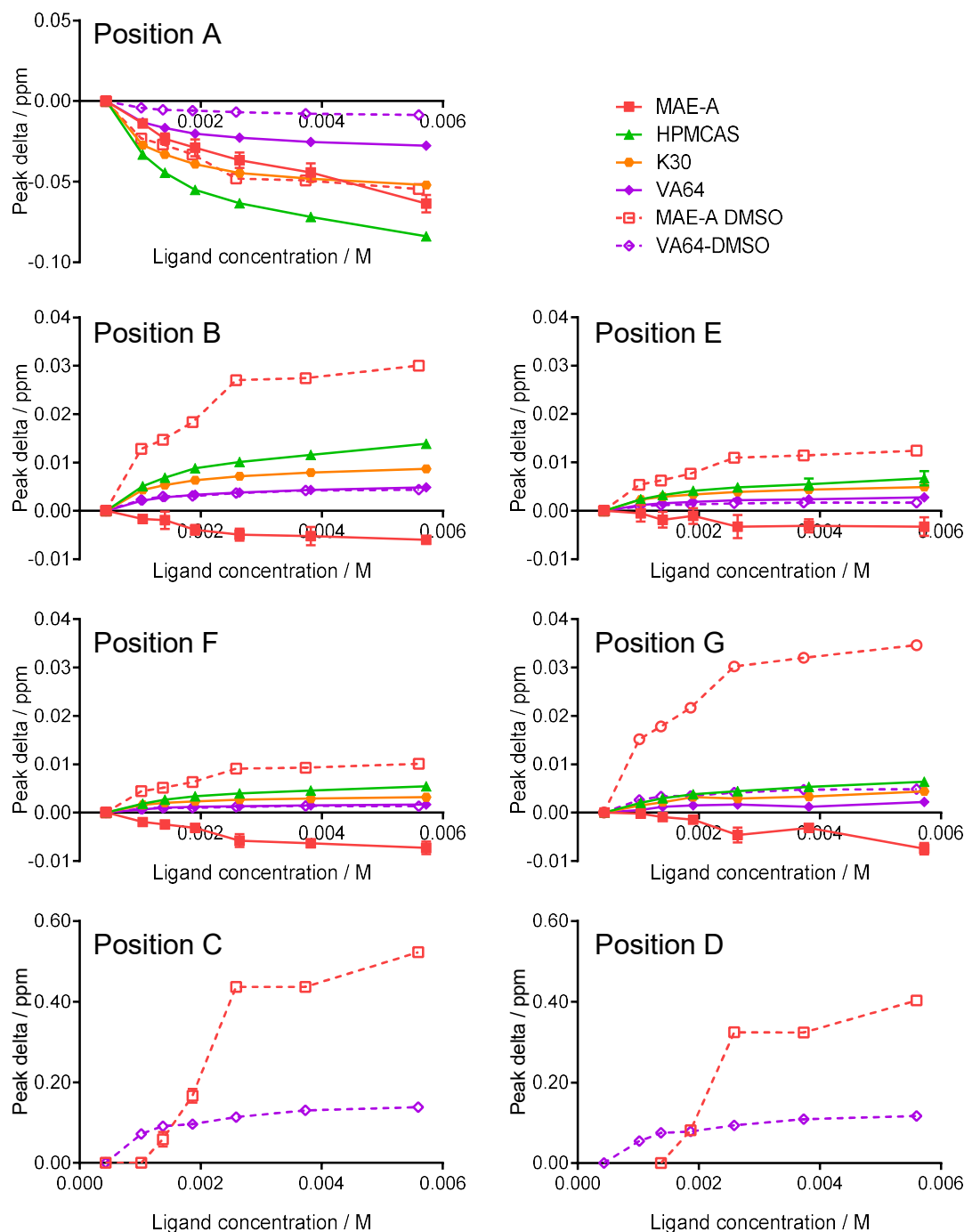


Figure 4.46 Binding curves for different proton positions of trimethoprim in titration experiments with various polymers (MAE-A, HPMCAS, VA64, K30) in MeOD. Only MAE-A and VA64 were tested in DMSO-d₆.

For the other polymers, the largest effects were observed at positions B and G. Position B was slightly more pronounced in methanol-d₄, while both positions performed equally in DMSO-d₆. Analysing the chemical structure, steric hindrance effects seem unlikely as an explanation, as both are comparable. Instead, position G is centred between both aromatic ring systems, reflecting a high influence in electron density distribution by a positive inductive effect, meaning that the electron density is reduced on position G. This makes the protons more accessible for interactions and

therefore, stronger peak shifts. The effect is enhanced in solvents with polarity as methanol compared to DMSO.

Figure 4.48 shows the calculated Scott-, Scatchard- and Benesi-Hildebrand plots for positions B, G, C, and D, with C and D being recorded only in DMSO-d₆ and only for MAE-A and VA64. For positions B and G, all plots lead to linear correlations with acceptable fits. For VA64, the curves in MeOD and DMSO are not overlapping at position G, but position B is comparable in both solvents. Position G is more electron shielded; therefore, the polarity of the solvent has a larger effect on the peak shift.

For positions C and D, good linear fits were obtained with titration data from VA64. The results from the MAE-A titration did not lead to a correlation of the data. More outliers with larger deviations are detected, leading to a negative slope in the Scott-plot and no pattern in the Scatchard-plot. In the binding curves it can already be seen that a typical curve shape is obtained with VA64, but not with MAE-A. Interactions seem to be significantly stronger here, but by this, also more diffuse.

To compare the outcome of the titration experiments, the results for the maximum possible peak shift, δ_{max} , were added for positions B and G and summarised in Figure 4.47. The three models lead to consistent results. MAE-A in methanol-d₄ was excluded from the evaluation due to the effects discussed above. Still, with MAE-A examined in DMSO-d₆, the ranking was MAE-A > HPMCAS > K30 > VA64 (both MeOD and DMSO). HPMCAS can interact with both hydrogen bonding and hydrophobic domains. As trimethoprim is less hydrophobic than cinnarizine, the more hydrophilic K30 works better compared to VA64 in this case.

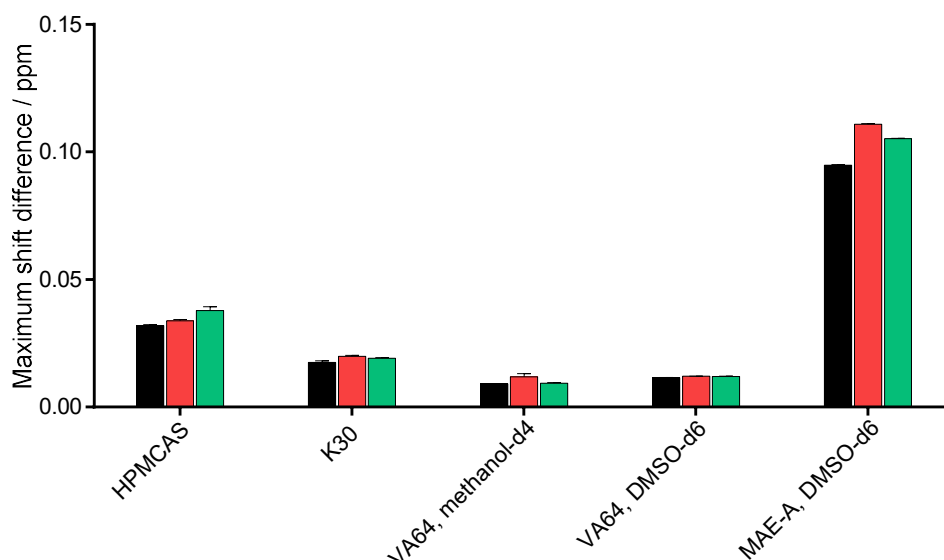


Figure 4.47 Maximum possible peak shift difference, δ_{max} , for proton positions B+G of trimethoprim in dependence of the polymer. The three applied models for calculation are Scott (black), Scatchard (red), and Benesi-Hildebrand (green).

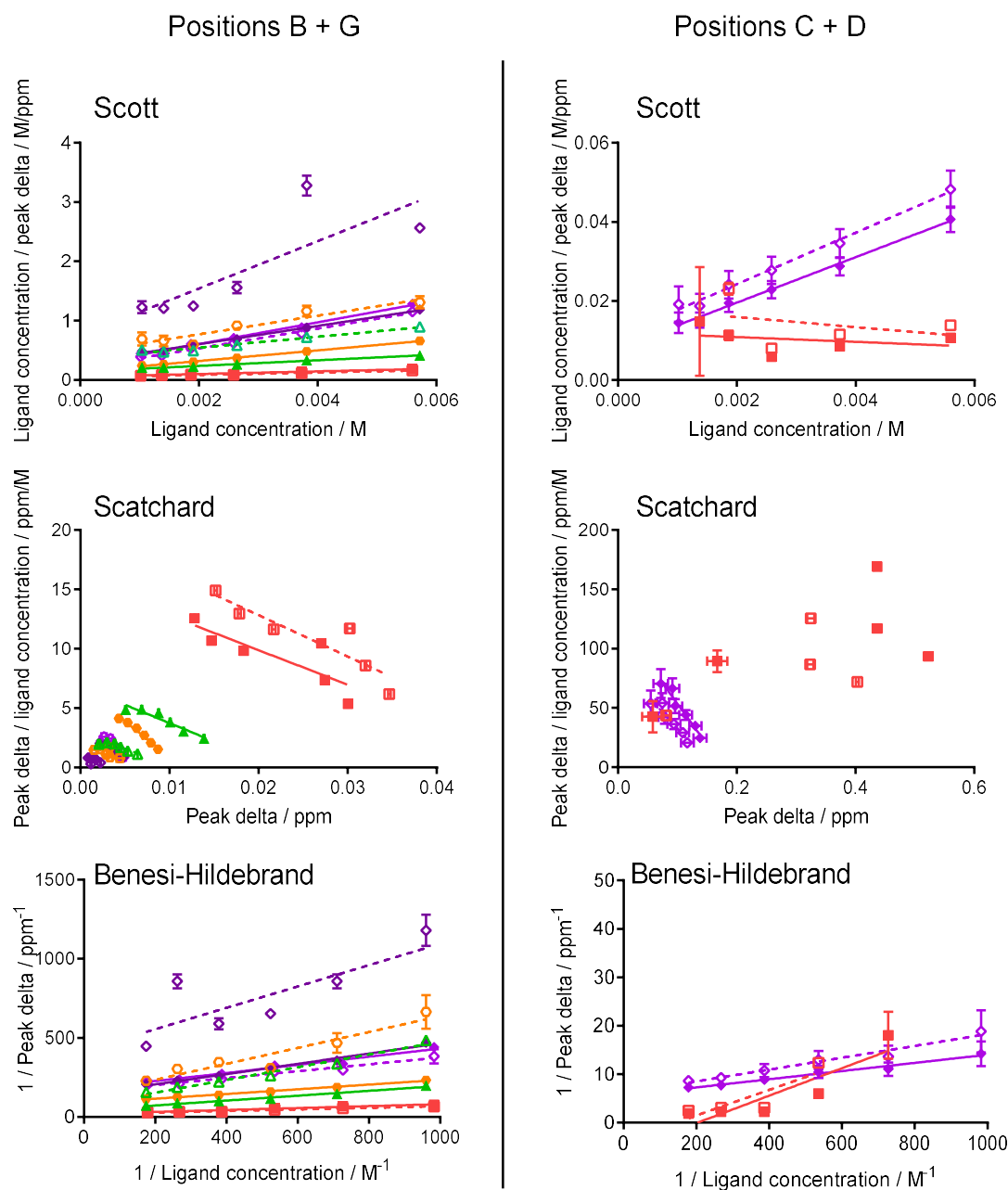


Figure 4.48 Scott-, Scatchard- and Benesi-Hildebrand-plots for proton positions B + G and C + D of trimethoprim. Shown are the plots for titrations with MAE-A (DMSO, red squares), HPMCAS (MeOD, green triangles), VA64 (DMSO, purple rhombuses and MeOD, dark purple rhombuses) and K30 (MeOD, orange hexagons). Positions B and C are filled symbols with solid lines; positions G and D are empty symbols with dashed lines.

4.3.15 Solubility parameters

The differences between solubility parameters of trimethoprim and the tested polymers are summarised in Table 4.22. With trimethoprim, the overall comparison with the polymers' solubility parameters is consistently unfavourable, with most of the outcomes being either poor or extremely poor. The comparison with PAA and PSSA resulted in favourable $\Delta\delta_e$ values, indicative of the potential for robust hydrogen bonding. The $\Delta\delta_{vdW}$ component exhibited poor compatibility,

attributable to their high polarity. By contrast, VA64 and K30 lack polar functionalities capable of interacting with trimethoprim, resulting in extremely poor $\Delta\delta_e$ and $\Delta\delta_{total}$ values. EPO displays the weakest predicted miscibility of all polymers tested. Trimethoprim also exhibits poor matching with MAE-A, HPMCAS, and HPMC. Following this model, none of the polymers would be expected to form stable, miscible systems with trimethoprim.

Table 4.22 Differences between the solubility parameters for trimethoprim and a selection of polymers. Given is the absolute value of the difference. The overall solubility parameter for crystalline trimethoprim is 25.21, the van der Waals solubility parameter is 18.80, and the electrostatic solubility parameter is 16.79.

	$\Delta\delta_{total}$ [MPa ^{1/2}]	$\Delta\delta_{vdw}$ [MPa ^{1/2}]	$\Delta\delta_e$ [MPa ^{1/2}]
MAE-A	10.90	8.03	7.38
EPO	12.18	6.61	12.17
HPMCAS	7.77	5.93	5.03
HPMC	7.52	5.69	4.90
VA64	10.27	5.20	10.62
K30	10.00	4.99	10.42
PAA	5.33	5.97	1.60
PSSA	6.26	6.06	2.77
Good (0 – 2)	Fair (2 – 5)	Poor (5 – 9)	Extremely poor (> 10)

The model for the solubility parameters applied in the MS Maestro software is based on the Hildebrand solubility parameter, which is most suitable for simple, non-polar molecules. The Hansen solubility parameter is also a measure of polarity and, as such, represents a higher degree of complexity. Trimethoprim itself possesses a higher polarity, therefore a comparison with the polymers based on the Hansen solubility parameter would be more suitable. The disadvantage of this approach is that it would require a substantially greater degree of computational capacity. As previously stated, the solubility parameters calculated in MS Maestro should be regarded as a preliminary indication of potential compatibility. For cinnarizine, a non-polar molecule, the results demonstrated a high degree of consistency with experimental findings on embedding and stability. For trimethoprim, the overall ranking that was found based on the solubility parameter differences was PAA < PSSA < HPMC = HPMCAS < K30 = VA64 < MAE-A < EPO. Though the absolute values for the differences show poor compatibility across all systems, the ranking appears to align with the findings from the embedding experiments in melt quenching and hot melt extrusion for the polymers that were used in these experiments (namely HPMC, HPMCAS, VA64 and EPO).

4.3.16 pK_a calculations

The percentages of different protonated states of trimethoprim at pH 6.8 are depicted in Figure 4.28 in chapter 4.2.12. One of the two sites is slightly more acidic when protonated, which lowers its probability of protonation at neutral pH. If one nitrogen becomes protonated, the second behaves as a very strong acid and is fully deprotonated under physiological conditions. Thus, doubly protonated molecules do not occur. The primary amine positions exhibit negative pK_a-values and therefore do not contribute to protonation under these conditions. At pH 6.8, approximately 90% of

trimethoprim molecules are deprotonated, making the molecule predominantly neutral in solution. Given this, ionic interactions with the polymers are unlikely, and no enhancement of solubility through ion pair formation is expected.

At position 1, being the nitrogen atom between the two primary amines, the pK_a is 4.11 and therefore slightly lower in comparison to position 2 with 5.86 (for the assignment of the position numbers, see Figure 3.1). While both primary amines exhibit electron-donating behaviour via a +I-effect (inductive) and a +M-effect (mesomeric/resonance) due to their conjugation with the aromatic system, these effects remain relatively weak overall as electron density is delocalised across the aromatic ring. While the combined electron-donating effects increase electron density at neighbouring atoms, they are outweighed by steric hindrance, thus rendering protonation at the outer amine position (position 2) more favourable.

4.3.17 Molecular modelling

In molecular modelling studies, trimethoprim was combined with selected polymers in drug loads of 10%, 30%, 50%, 70%, and 90% (w/w). The number of interaction events was counted for both API-polymer- and API-API-interactions. In Figure 4.49, the combined interaction counts (hydrogen bonding and π - π -stacking) for API-polymer- and API-API-interactions are plotted. The maximum recorded for API-polymer-interactions was observed to be highest with PAA, PSSA and MAE-A, followed by HPMC and HPMCAS. Polymer chains interpose between API molecules and thereby disrupt drug-drug contacts.

Hydrogen bonding is the primary interaction between trimethoprim and the polymers, therefore EPO demonstrated a lower efficacy in comparison to its combination with cinnarizine, as evidenced by a reduced AUC. VA64 and K30 performed similar with trimethoprim and cinnarizine. While there were more possibilities for polar interactions than with cinnarizine, the benefit of non-polar interaction ceased with the more polar molecule trimethoprim. The order of all three categories (total AUC, ABC, and drug load intercept, all summarised in Figure 4.50) was PAA > PSSA > MAE-A > HPMC > HPMCAS > VA64 > K30 > EPO.

As mentioned above and in contrast to the outcomes observed for cinnarizine, the curves for API-API-interactions differ for different polymer combinations with trimethoprim. In case of cinnarizine, π - π -stacking was the only interaction detected in MS Maestro. However, trimethoprim molecules had also been observed to form hydrogen bonds without the need for polymer addition. Figure 4.51 compares hydrogen bonding and π - π stacking among trimethoprim molecules in the presence of the different polymers. π - π stacking was only minimally affected by polymer type, whereas hydrogen bonding varied substantially. PAA, PSSA, and MAE-A disrupted intramolecular hydrogen bonding most effectively, suggesting that polymer polarity rather than molecular size or steric properties plays the dominant role in suppressing API-API interactions. Due to more interactions between API and polymer, the API-API-interactions are disrupted.

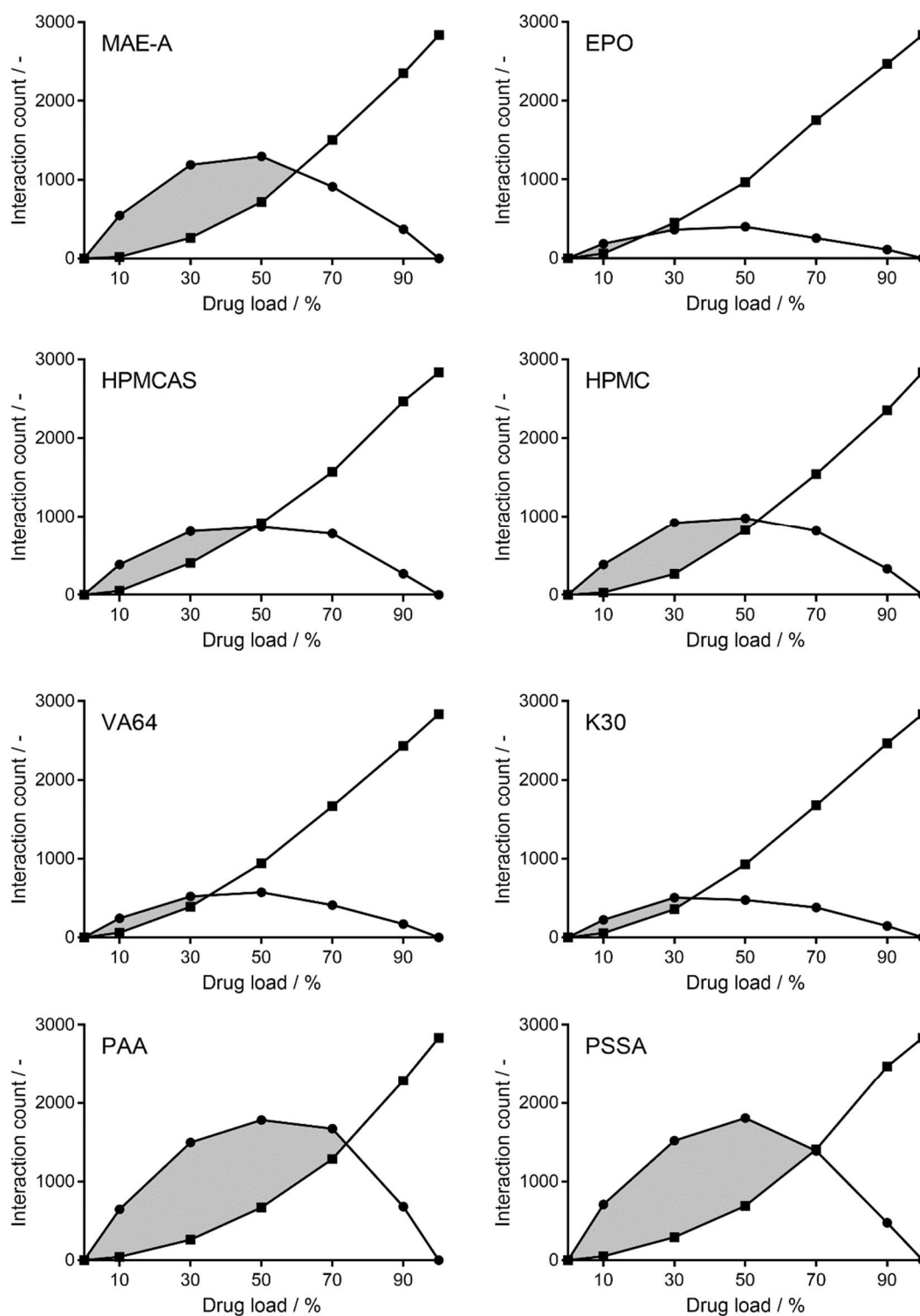


Figure 4.49 Combined interaction counts (hydrogen bonding + π - π -stacking) for trimethoprim-polymer combinations with MAE-A, EPO, HPMCAS, HPMC, VA64, K30, PAA, and PSSA. Shown is the number of API-polymer-interactions (circles) and API-API-interactions (squares); the grey area marks the range where API-polymer-interactions outnumber API-API-interactions.

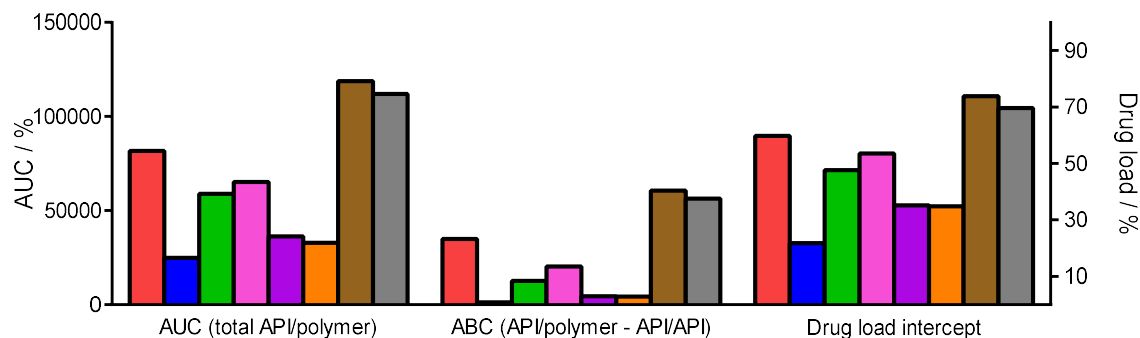


Figure 4.50 Area under the curve (AUC), area between the curves (ABC) and drug load intercept for API-polymer systems with trimethoprim. Shown are results for MAE-A (red), EPO (blue) HPMCAS (green), HPMC (orange), VA64 (purple), K30 (pink), PAA (grey), and PSSA (black).

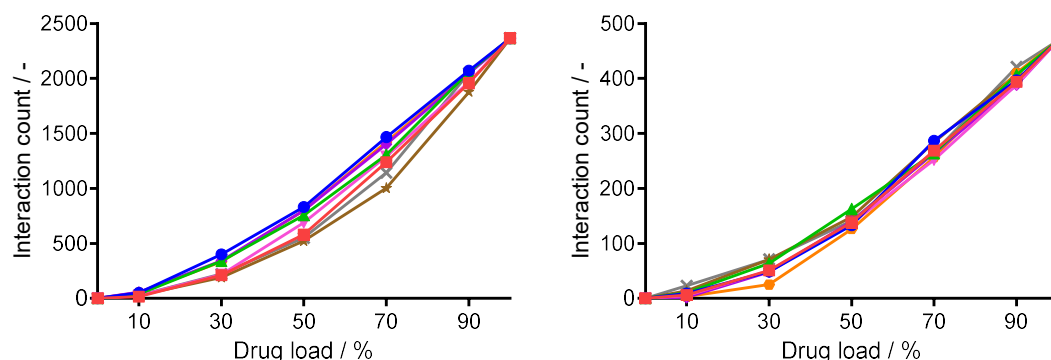


Figure 4.51 Interaction count for trimethoprim-polymer combinations. Left: sum of aliphatic and aromatic hydrogen bonding; right: π - π stacking. The polymers shown are MAE-A (red squares), EPO (blue circles) HPMCAS (green triangles), HPMC (pink inverted triangles), VA64 (purple rhombuses), K30 (orange hexagons), PAA (brown stars) and PSSA (grey crosses).

In the “500+1”-modelling experiments with trimethoprim, the ranking of the polymers regarding interactions per monomer was PSSA > HPMC > HPMCAS > PAA > MAE-A > VA64 > K30 > EPO (see Table 4.23). As trimethoprim is polar and can form more hydrogen bonds than cinnarizine, it seems that more interactions can be formed with a structure moiety that is more polar itself. This rational was also seen when comparing the interaction counts for the polymers between cinnarizine and trimethoprim. For MAE-A, HPMCAS, HPMC, PAA and PSSA, more interactions were found with trimethoprim, whilst the less polar polymers EPO and K30 displayed larger interaction counts with cinnarizine, both per monomer and in total. For VA64, the difference was negligible.

When calculating the interaction count per real size molecule based on the downsizing factor, the ranking found was MAE-A > PAA > PSSA > HPMC > VA64 > HPMCAS > K30 > EPO. As only MAE-A and HPMCAS were used with a different downsizing factor than the other polymers, the order stays the same except for these two polymers. Owing to its high molecular weight and the resultant large quantity of monomer units, interactions with MAE-A predominate over those with

alternative polymers. By contrast, the situation is the opposite for HPMCAS with low molecular weight. When considering the interaction count per gram of polymer substance, the ranking order is changed to PAA > PSSA > MAE-A > HPMC > HPMCAS > VA64 > K30 > EPO. Acidic polymers dominate the interactions with trimethoprim. EPO is ranked at the end of the sequence. As explained in chapter 4.2.13, the calculation does not provide the actual number of interactions in a laboratory setting, as the environmental factor can significantly influence molecular structures and synergies.

Table 4.23 Interaction count per monomer obtained in “500+1”-experiments for trimethoprim with chosen polymers.

	MAE-A	EPO	HPMCAS	HPMC	VA64	K30	PAA	PSSA
Interactions per molecule	212	9	154	81	33	14	236	131
Interactions per monomer	1.14	0.23	1.97	2.13	0.58	0.32	1.71	3.20
Interactions per real molecule (multiplied by downsizing factor)	4240	90	154	810	330	140	2360	1310
Interactions · 10 ²¹ per g of polymer	7.95	1.15	5.44	5.79	3.44	1.71	14.16	10.48

4.3.18 Summary for trimethoprim formulations

Trimethoprim, a class I compound and therefore a fast crystalliser regarding the classification of Baird et al. [59], showed complex behaviour in terms of residual crystallinity and amorphous embedding depending on the processing route and polymer matrix. Across all investigated systems, HME generally yielded lower crystallinity than spray drying, with melt quenching serving as a reference that consistently produced the fewest residual crystallinity. A summary of tested characteristics together with a ranking of the polymers delivering a beneficial effect is given in Table 4.24. For certain factors, MAE-A and K30 are not listed, as no residual crystallinity could be determined.

At a drug load of 50% (w/w) in HME, the order of residual crystallinity was HPMC < HPMCAS < VA64 < EPO, whereas in spray drying the order shifted to HPMCAS < VA64 < HPMC < EPO. This ranking indicates that while celluloses such as HPMC and HPMCAS can embed a substantial amorphous fraction, the embedding capacity is strongly dependent on the preparation technique and the inherent polymer characteristics. For example, in HME with 10% (w/w) drug load, EPO displayed lower crystallinity than HPMC and VA64, which can be attributed to its very low glass transition temperature and melt viscosity. These characteristics facilitated early softening of the polymer as well as prolonged softening during extrusion, which enabled trimethoprim to be better distributed throughout the polymeric matrix, which also helped mitigate the potential for crystal

clustering. However, EPO did not perform well overall with higher drug loading due to its basic functional groups that cannot form stable molecular interaction with the weak base trimethoprim.

Table 4.24 Overview of polymer rankings according to the methods applied in this study for trimethoprim. Beneficial effects or performance regarding the respective characteristic decrease from left to right (such as lower degree of drug crystallisation, higher amount of dissolved drug, and more possible drug-polymer interactions). The colours indicate the type of functional groups: acidic (grey), neutral (white), basic (black); if two polymers of different groups performed similar, they are marked as light grey.

		Beneficial effects				
Residual crystallinity	50% ASDs SD	HPMCAS	VA64	HPMC	EPO	
	10% ASDs HME	HPMCAS	EPO	HPMC = VA64		
	50% ASDs HME	HPMC	HPMCAS	VA64	EPO	
Embedding capability	HME	HPMCAS	HPMC	EPO	VA64	
	Spray drying	EPO	VA64	HPMCAS	HPMC	
	Melt quenching	HPMC = HPMCAS	VA64	EPO		
	High throughput screening	MAE-A	HPMCAS	VA64 = K30	HPMC	EPO
Flory-Huggins	χ	HPMC	HPMCAS	VA64	EPO	
Dissolution	AUC 10% milled extrudates, 200 mg	K30 = VA64	MAE-A	EPO	HPMCAS	
	AUC 10% milled extrudates, 2000 mg	K30	VA64	EPO = MAE-A = HPMCAS		
	AUC 50% milled extrudates, 2000 mg	MAE-A	EPO	K30	VA64	HPMCAS
	AUC TFAM 30% spray-dried samples	HPMCAS	VA64	K30	HPMC	MAE-A
	Dissolution speed of polymers	VA64	K30	HPMC	MAE-A	HPMCAS
					EPO	
1H-NMR	δ_{max}	MAE-A	HPMCAS	K30	VA64	
Modeling	$\Delta\delta_{total}$ (solubility parameter)	HPMC = HPMCAS	K30 = VA64	MAE-A	EPO	
	AUC (total API/polymer)	MAE-A	HPMC	HPMCAS	VA64	K30
	ABC (API/polymer – API/API)	MAE-A	HPMC	HPMCAS	VA64	K30
	No. interactions per monomer	HPMC	HPMCAS	MAE-A	VA64	K30
	No. interactions per molecule; calculated for real size	MAE-A	HPMC	VA64	HPMCAS	K30
	No. interactions per 1 g of substance	MAE-A	HPMC	HPMCAS	VA64	K30

The embedding efficiency differed not only across polymers but also between processes. In HME, VA64 exhibited the lowest capacity to stabilize trimethoprim in the amorphous form, whereas HPMCAS provided the most effective embedding, followed by HPMC and EPO. In contrast, the ranking in spray drying was HPMC < HPMCAS < VA64 < EPO, a sequence reflecting the impact of drying kinetics and solvent interactions. Celluloses such as HPMC and HPMCAS embedded trimethoprim less effectively in spray drying due to their longer drying times and tendency to retain residual solvent [190], which hindered complete amorphisation. However, crystallinity could be

reduced by increasing the inlet temperature during spray drying for cellulose-based carriers, since faster solvent removal promoted a higher amorphous fraction. Melt quenching in the DSC confirmed the superior embedding ability of celluloses, ranking HPMC = HPMCAS > VA64 > EPO, but highlighted that solvent interference can undermine these advantages. In the high-throughput screening, HPMC performed worse than expected from the results of the melt-based manufacturing techniques due to the formation of highly viscous systems upon drying with a reduced drying kinetic. Milling, by contrast, was ineffective in embedding trimethoprim into any polymer system, as no relevant amorphous fractions could be stabilised.

When considering the drug load, there was a consistent trend: at 50% drug load, the celluloses showed the highest performance in both HME and SD, while EPO was the poorest performer. This finding was congruent with the anticipated results based on interaction potential, as the celluloses have the potential to form stabilising hydrogen bonds and hydrophobic contacts, with limited beneficial interactions for EPO. The ternary system of HPMCAS/VA64 1:1 showed a lower crystallinity level than the corresponding binary systems at 50% drug load. At 70% drug load, VA64 was tested at a binary level, with a higher amount of crystallinity than seen for the ternary mixtures. Overall, this finding illustrates that polymer blending can be a useful strategy for decreasing the degree of residual crystallinity and improving physical stability. However, the dissolution performance was unaffected compared to the binary VA64 system.

At 70% drug load in spray drying, approximately half of the included trimethoprim could be stabilised amorphously with the cellulose ethers, resulting in about 35% amorphous drug load in the system. Even at lower total drug loads (30% (w/w)), not all the included trimethoprim could be embedded amorphously, with crystalline fractions often accounting for roughly half of the API. This indicates that while cellulose ethers can host a relatively high amorphous fraction, small inhomogeneities or the presence of a crystal seed can trigger large-scale crystallisation.

The correlation between the empirical results obtained by melt quenching, HME and spray drying on the one hand and molecular modelling on the other hand revealed that the stabilising capacity of these polymers is driven primarily by their ability to disrupt drug-drug interactions through hydrogen bonding. HPMCAS and HPMC outperformed other carriers in melt quenching and HME, while EPO and VA64 struggled to prevent recrystallisation. This experimental ranking is almost perfectly mirrored by the molecular simulations, where the "interaction landscape" favoured acidic and neutral celluloses over basic or neutral synthetic polymers. The modelling explains this by showing that trimethoprim's strong crystallisation drive is fueled by its own hydrogen-bonding potential. Polymers like HPMCAS and MAE-A succeed because they interpose their own polar functional groups between drug molecules, effectively out-competing the drug's affinity for itself. The poor performance of EPO in HME, despite its low glass transition temperature suggesting good processability, is explained by its lack of favourable hydrogen-bonding sites, which the simulations identified as the dominant stabilisation mechanism for trimethoprim.

The prediction of drug-polymer compatibility using solubility parameters was of limited value for trimethoprim, as the Hildebrand parameter provides a better fit for nonpolar compounds such as cinnarizine, while trimethoprim is considerably more polar. Within this framework, only PAA and PSSA appeared favourable considering polar interactions and the potential for hydrogen bonding. Overall, the model did not capture the behaviour of trimethoprim adequately. A Hansen solubility parameter approach would likely provide more reliable predictions, but at a higher computational cost. pK_a estimations showed that trimethoprim remains mostly neutral, meaning deprotonated, at pH 6.8, indicating that ionic interactions with polymers are unlikely.

Dissolution studies revealed a strong influence of drug load and polymer type on the drug release. At low drug loads (10%), the dissolution rate reflected primarily the intrinsic dissolution speed of the polymer, with HPMCAS performing worst and VA64 and K30 best. At higher drug loads (50%), interaction capacity became more decisive, as shown by the superior performance of MAE-A. The fact that MAE-A outperformed others at high loads, despite showing crystallinity, suggests that specific molecular interactions are more vital for stabilising supersaturation than maintaining a purely amorphous state. This decoupling of crystallinity and performance is a crucial takeaway. It implies that an amorphous system like the HPMCAS 10% sample can still fail if the polymer cannot facilitate rapid release or prevent the drug from becoming "trapped" in a slow-dissolving matrix.

In the TFAM setup, the membrane acts as a selective barrier that reveals the "quality" of the dissolved drug. With MAE-A, it was found that the concentration was higher in the interaction compartment compared to the permeate, suggesting a retention of API molecules by interactions with the polymer. While these complexes help achieve high concentrations in a standard beaker test, they are effectively too bulky to pass through biological-style membranes. This underscores a trade-off in formulation: the very interactions that stabilise a drug in solution can, if too strong, inadvertently reduce its bioavailability by preventing it from crossing the membrane. For HPMCAS, the AUCs were higher for small drug loads, supporting the often described "falling off the cliff"-effect at decreased polymer-API ratios [18,36,159].

A mismatch was found between the predicted ranking by molecular modelling and the observed dissolution rates for K30 and VA64. While the modelling approach ranked these polymers poorly due to limited molecular interactions, dissolution testing in the USP II apparatus showed they achieved the most rapid drug release at low loads (10%). This suggests that in the early stages of dissolution, the intrinsic solubility and hydration rate of the polymer carrier are more influential than the strength of the drug-polymer molecular bond. However, at higher drug loads (50%), the modelling became more predictive: the lack of strong interactions in VA64/K30 led to an effect where they could no longer stabilise supersaturation at levels as high as MAE-A, a high-interaction polymer, did. The modelling's "interaction landscape" predicted that acidic polymers (like MAE-A and HPMCAS) would outperform basic or neutral polymers by out-competing the drug's affinity for

itself, thereby disrupting the drug-drug contacts that lead to recrystallisation, though this prediction was only valid for high drug loads.

NMR titration studies in methanol-d₄ and DMSO-d₆ indicated strong molecular interactions, ranking MAE-A > HPMCAS > K30 > VA64, with HPMCAS standing out for its dual ability to engage in hydrogen bonding and hydrophobic interactions. HPMC and EPO were not tested in this experiment. Molecular modelling further supported these observations, ranking the polymers in terms of per-mass interactions with trimethoprim as MAE-A > HPMC > HPMCAS > VA64 > K30 > EPO. The pronounced advantage of acidic polymers reflects trimethoprim's polarity and high hydrogen bonding capacity. EPO, in line with experimental findings, consistently ranked lowest, underscoring its limited interaction potential with trimethoprim despite favourable processing properties.

Overall, trimethoprim's formulation behaviour highlights the interplay between molecular interactions, polymer properties, and processing pathways, with celluloses standing out as the most reliable carriers despite their challenges in solvent-based methods, while EPO remains a paradoxical case of favourable processability in melting processes but poor stabilisation at higher drug loads. It was revealed that while molecular dynamics can guide polymer selection based on chemical affinity, it fails to account for processing-induced variables. In dissolution testing, HPMCAS showed varying performance based on whether it was spray-dried or extruded. In general, the dissolution behaviour can be predicted in a more appropriate manner by different modelling approaches as dissipative particle dynamics by coarse-grained modelling [191].

5 Summary and conclusion

5.1 Comparison between the active ingredients

Comparing trimethoprim and cinnarizine, it was found that small differences in molecular design lead to a substantially different formulation behaviour preparing amorphous solid dispersions. Trimethoprim has a higher aqueous solubility at a neutral pH than cinnarizine, but trimethoprim does not show the same level of pH-dependence of its solubility. In comparison to trimethoprim, cinnarizine has a more dramatic shift in solubility with a decreased pH due to its considerably greater protonation tendency. This difference has immediate consequences with respect to formulation. Cinnarizine formulations can employ pH-responsive polymers to achieve larger solubility increases, whereas trimethoprim does not respond in the same way.

The two drugs' solvent partitioning behaviour is reflective of their intrinsic polarity. Both drugs dissolve fairly well in methanol. However, the poorer octanol solubility of trimethoprim relative to cinnarizine indicates that trimethoprim is more polar than cinnarizine. This polarity has implications for the membrane permeability but also leads to differences in the categories of polymer interactions that are possible. The higher polarity of trimethoprim also means more opportunities for hydrogen-bonding interactions with acidic polymers. Cinnarizine interacts more effectively with hydrophobic and less polar matrices compared to trimethoprim, with still good results for combinations with acidic polymers.

For cinnarizine, up to thousand-fold solubility increases can be achieved when formulated with the appropriate polymers. Trimethoprim rarely exceeds a two-fold solubility enhancement, with no polymer achieving more than a ten-fold increase. This difference emphasises the limited effect of solubility-enhancing polymers for trimethoprim. However, there is some benefit with PAA, PSSA, and MAE-A, which suggests that acidic functional groups can produce a pH shift large enough to improve the solubility slightly. Cinnarizine has a much broader range of polymer effects, allowing for a clear ranking of excipients, whereas trimethoprim has a narrower range making any ranking of effects more difficult to ascertain.

The differences in polymer embedding likewise emphasises the different physicochemical natures of the two APIs. Cinnarizine shows a benefit from the high molecular mobility of EPO during processing, which contributes to better incorporation in polymer matrices, even without molecular interactions. For trimethoprim, more substantial stabilisation during cooling in hot melt extrusion or solvent evaporation in spray drying is required to avoid crystallisation. This is consistent for trimethoprim being classified as rapid crystalliser versus cinnarizine being an intermediate crystalliser. Interestingly, phase diagrams indicate that trimethoprim gains a more stable amorphous state compared to cinnarizine because of having a higher glass transition temperature. This is a significant observation when considering long-term stability and shelf-life. Although the cinnarizine formulations may look more promising from the outset, they have a higher risk of instabilities.

Overall, cinnarizine is embedded more effectively in a range of polymers, benefiting from hydrophobic interactions with cellulose derivatives and povidones. Trimethoprim relies more on polar interactions with acidic polymers, where hydrogen bonding reduces self-association and lowers crystallisation tendencies. The type of polymer is therefore critical for trimethoprim, while cinnarizine is more broadly compatible with hydrophobic carriers.

Ionisation further strengthens these observations. Cinnarizine exhibits greater protonation in solutions with a neutral pH, and increases the ionic interactions, resulting in an increased strong pH-dependent solubility. This mechanism cannot be used for trimethoprim as effectively, and its stabilisation occurs through specific hydrogen bonding. Interaction analyses confirm this mechanistic interpretation. Trimethoprim forms more interactions with polar types of polymers such as MAE-A, HPMCAS, HPMC, PAA, and PSSA, while cinnarizine forms greater interactions with the hydrophobic polymers, for example, EPO, and K30. Also, interaction types differ, which is particularly evident in the case of the celluloses. Trimethoprim can form hydrogen bonds and polar interactions, while cinnarizine interacts with the hydrophobic domains. VA64 is a borderline case, with negligible differences between the two APIs.

The production method contributes to the overall residual crystallinity, and the trends are similar across both APIs. Melt quenching produced the fewest amount of crystallinity, followed by hot melt extrusion, with spray drying generally leaving the highest degree of crystallinity. While some outliers exist, this ordering reflects fundamental differences in cooling rates, residual solvents, and molecular mobility between the processes. Importantly, the shared trend indicates that processing effects are API-independent, but the absolute level of crystallinity was still depending on the specific drug-polymer combination.

The dissolution behaviour further shows the compound-specific interplay between drug properties and polymer functionality. For both trimethoprim and cinnarizine, under low drug-loaded conditions, their dissolution can mostly be characterised by the dissolution of the polymer carrier, indicating a carrier-controlled release mechanism. Furthermore, at this lower drug-loaded condition, the intrinsic differences between the APIs become less apparent and their interactions with the polymer are less important. However, as the drug loading increases, the influence of the polymer functional groups is more apparent. In dissolution testing, neither trimethoprim nor cinnarizine appears to experience any considerable dissolution advantage by being embedded in basic polymers, which may result from having no stabilising interactions within the API-polymer system. Cinnarizine demonstrates enhanced dissolution with both neutral and acidic polymers, consistent with its hydrophobic and protonated character, which allows stabilisation through a combination of hydrophobic and ionic interactions. Neutral polymers lead to a more rapid dissolution rate at first with subsequent parachute effects, while acidic polymers tend to release the API slower but more consistent. Trimethoprim, by contrast, benefits from acidic polymers because they can engage in strong hydrogen bonding and polar interactions that limit self-association and recrystallisation tendencies during release.

Finally, ternary formulations show inconsistent results. For cinnarizine, introducing a second polymer was disadvantageous, destabilising the amorphous phase and offering no benefit in dissolution properties. In contrast, trimethoprim benefited from ternary systems in terms of reduced crystallinity and improved physical stability, although the dissolution performance itself remained largely unaffected. This suggests that ternary formulations for trimethoprim should be considered where long-term stability is a priority.

Overall, these findings emphasise that trimethoprim and cinnarizine represent two fundamentally different formulation challenges, though both are poorly soluble weak bases classified as BCS class II drugs. Cinnarizine is highly responsive to a wider polymer selection and benefits substantially from solubility enhancement strategies, but at the cost of long-term stability for neutral polymers. Trimethoprim is less responsive to solubility-enhancing polymers and requires more stabilisation during processing, yet it offers better stability once embedded. The contrast between the two drugs demonstrates that successful ASD design depends less on universal “best” polymers or methods and more on a careful alignment between the molecular properties of the API and the functional groups of the excipient.

5.2 Relevance of the analytical and predictive methods

The central questions of this work were (i) whether amorphous solid dispersions can be stabilised in the amorphous state over time, (ii) whether their dissolution performance can be enhanced compared to the crystalline active pharmaceutical ingredient, and (iii) which analytical methods can best model or predict the outcomes thereof. ASDs were produced by melt quenching, spray drying, and hot-melt extrusion. A broad range of analytical and predictive methods was applied to characterise both the amorphous stability and the dissolution behaviour. The collective outcomes highlight the complementary nature of these methods and the need for a multi-factorial evaluation strategy.

Measuring residual crystallinity after manufacturing and storage was vital to understanding kinetic stabilisation. Initial amorphous products rarely recrystallised entirely, but any residual crystallinity present immediately after processing tended to grow during storage, confirming its role as a nucleation source. These findings aligned well with embedding capacity assessments, where polymers that maximised amorphous incorporation of API also reduced crystallisation during storage. High-throughput screening of embedding capacity further demonstrated its usefulness as an early predictive tool for stability ranking, though the exact amount of embeddable API was not predictable due to different material amounts and process durations.

Predictive phase diagrams allowed estimation of the theoretical amorphous stabilisation limit. For cinnarizine, the experimental results closely matched the theoretical predictions; however, trimethoprim displayed faster crystallisation kinetics, making stabilisation less favourable than

predicted. This reinforces that phase diagrams provide valuable directional insights, but do not include other influencing factors as initial crystallinity or environmental stress as increased humidity.

Gordon-Taylor modelling and the resulting deviation (Δk) between theoretical and experimentally-derived curves offered an additional perspective on polymer-API interactions. Δk effectively differentiated between the best and worst performing polymers, outperforming the AUC as predictive indicator. In contrast, Flory-Huggins interaction parameters contributed limited predictive value in this study, likely due to the inability to include low drug loads and the inherent assumptions about ideal mixing. Similarly, improvements in aqueous solubility upon polymer addition were not predictive of stability or dissolution outcomes.

Dissolution testing, including milled extrudates and spray-dried samples at different drug loads, vacuum-compression-molded discs, and a TFAM-setup, revealed that dissolution success was less dependent on polymer-API interactions and more strongly influenced by polymer dissolution rate and the formulation's specific surface area. Polymer behaviour in water, especially the formation of gels or diffusion layers, played a critical role and clearly decoupled dissolution performance from amorphous stability.

¹H-NMR titrations provided a more direct measure of interaction strength, with maximum peak shifts reflecting stabilising interactions, particularly relevant for acidic polymers with basic APIs. Molecular modelling offered early-stage predictions through Hildebrand solubility parameters and interaction counting. While solubility parameter calculations yielded only rudimentary predictions, interaction counting correlated well with both ¹H-NMR results and the experimentally observed amorphous stabilisation. Importantly, interaction numbers must be normalised by mass rather than by polymer chain length to avoid misleading polymer-size effects.

Overall, the results demonstrate that no single analytical or predictive method can answer both guiding questions simultaneously. Methods that correlate strongly with amorphous embedding and stability (e.g., residual crystallinity, embedding capacity, Δk from Gordon-Taylor, and interaction counting) are not the same methods that best explain dissolution performance, which is primarily driven by formulation geometry, including particle size, and polymer dissolution rate. API-polymer interactions are crucial, but not limited to simple acid-base contributions: hydrophobic interactions were shown to be equally important, as reflected in differences between structurally similar polymer pairs such as VA64 versus K30 or HPMCAS versus HPMC. Furthermore, the manufacturing technique itself affects the final behaviour of ASDs, particularly via the presence or absence of residual solvent during the drying process and its duration. Follow-up studies should focus on solvent effects as hydration kinetics of the ASD particles during dissolution or differences in stability due to organic solvents.

In conclusion, predicting the performance of ASDs requires a more complex and holistic understanding than any single analytical or theoretical approach can provide. The most reliable

strategy combines interaction-focused predictive methods for stability assessments, and dissolution studies. The interplay between kinetic stabilisation and dissolution enhancement remains system-specific.

5.3 Overall summary

This study examined the complex relationship between two active ingredients, namely cinnarizine and trimethoprim, and different polymers, and the influence of their molecular interactions on the performance of binary and ternary amorphous solid dispersions. Through a systematic evaluation of preparation methods such as melt quenching, hot melt extrusion and spray drying, together with analyses of residual crystallinity, phase diagrams, solubility enhancement, and NMR titrations, critical insights into the stabilisation of amorphous states and dissolution performance were elucidated.

The findings revealed that acidic polymers like MAE-A and HPMCAS demonstrated the most significant ability to stabilise the amorphous form in the solid state and to deliver the highest level of molecular interactions with the weak bases cinnarizine and trimethoprim as shown in ¹H-NMR titration. This method was applied on drug-polymer-systems for the first time. Besides acid-base interactions, the melting behaviour of the polymers including the glass transition temperature and melt viscosity had a crucial influence on the embedding of cinnarizine into the polymeric matrix. For the long-term stability, it was found that initially crystalline ASDs were prone to further crystallisation, while new crystallisation from a completely amorphous, homogeneous system was rare. The influence of drug load on the released amount of drug in dissolution testing was found to be consistent with recent studies on the so-called limit of congruence, up to which the polymer dictates the dissolution speed. In this study, this was the case for MAE-A, VA64, and K30 when comparing 10% (w/w) and 50% (w/w) drug load, for both cinnarizine and trimethoprim.

The findings further underscore that the choice of manufacturing method plays a decisive role not only in the formation of ASDs, but also in their stability and dissolution performance. Although both hot melt extrusion and spray drying were able to produce ASDs to varying extents, differences in residual crystallinity, dissolution behaviour, and stability were apparent across formulations. Hot melt extrusion enabled the formation of ASDs with higher drug loads and superior physical stability, particularly when polymers like HPMCAS or HPMC were used. Spray drying, in contrast, often resulted in higher residual crystallinity at equivalent drug loads, likely due to longer thermal exposure and potential residual solvent effects. Nevertheless, spray drying was able to generate trimethoprim formulations with rapid initial dissolution and high levels of supersaturation in the TFAM, particularly when VA64 or HPMCAS were used. These results highlight that formulation success is not solely dependent on achieving a fully amorphous product. Overall, neither HME nor spray drying offer a universally superior approach for ASD preparation. Instead, formulation performance depends on a combination of drug load, polymer selection, processing parameters, and the specific

goals of the formulation, whether that is maximising amorphous stability or enhancing absorption via rapid dissolution. The observed discrepancies between expected and actual dissolution behaviour further suggest that traditional markers of success, such as XRPD amorphicity, may not always correlate with in vivo-relevant performance. These insights highlight the complex interplay of polymer characteristics, processing methods, and molecular interactions and can guide formulation scientists in the rational design of ASD systems for poorly soluble drugs like cinnarizine and trimethoprim.

The combination of pK_a prediction, solubility parameter estimation, and molecular modelling offers a useful approach in understanding how APIs interact with different polymer environments. Predicted pK_a values help to explain the solubility behaviour at various pH levels and give insight into the likely protonation sites of a molecule, which cannot be directly obtained from laboratory experiments. For cinnarizine and trimethoprim, simulations showed that while full protonation of both basic sites is unlikely, cinnarizine tends to be more protonated overall compared to trimethoprim. Hildebrand solubility parameters, though quick and easy to compute, have their limitations. They perform well for non-polar compounds like cinnarizine, offering a good estimate of polymer compatibility. However, their application is limited with more polar molecules such as trimethoprim, as they mainly capture non-polar interactions and do not account for specific effects like proton transfer, ionic interactions, or specific hydrogen bonding.

Molecular dynamics simulations, particularly those involving large cells and “500+1” API-polymer systems, provide a more detailed insight. They show that in systems dominated by non-polar stacking interactions, the polymer content is the key factor in disrupting API-API interactions, not the chemical structure of the polymers. For polar APIs like trimethoprim, the functional groups of polar polymers can enhance hydrogen bonding with the API, thereby improving the compatibility and disrupting API-API bonding. Still, increased hydrogen bonding potential does not always translate to better performance, especially when there is a mismatch in polarity between the API and the polymer. For example, trimethoprim’s higher polarity can limit its compatibility with non-polar polymers, despite offering more potential interaction sites than cinnarizine. The “500+1” approach further allows comparison across polymer sizes and architectures, helping to identify optimal chain lengths for desired interaction profiles. This makes it a practical tool for guiding the design of new polymer excipients and improving formulation strategies.

Taken together, this study provides a detailed picture of the multifactorial determinants of ASD performance. The integration of experimental and computational approaches demonstrates how drug-polymer interactions, polymer physicochemical properties, manufacturing methods, and formulation design collectively shape stability and dissolution behaviour. Future work should build upon these insights to refine predictive tools, expand the scope of studied APIs, and tailor excipient design toward more robust and biorelevant amorphous formulations.

6 Kurzzusammenfassung

Diese Studie untersucht die komplexe Beziehung zwischen den Wirkstoffen Cinnarizin und Trimethoprim und verschiedenen Polymeren sowie den Einfluss ihrer molekularen Wechselwirkungen auf die Leistungsfähigkeit binärer und ternärer amorpher fester Dispersionen (ASDs). Durch eine systematische Bewertung von Herstellungsmethoden wie Schmelzabschreckung (melt quenching), Schmelzextrusion (HME) und Sprühtrocknung, ergänzt durch Analysen der Restkristallinität, Phasendiagramme und NMR-Titrations, werden wichtige Einblicke in die Stabilisierung amorpher Zustände und das Auflösungsverhalten gewonnen.

Die Ergebnisse zeigen, dass saure Polymere wie Hypromelloseacetatsuccinat und Methacrylsäure-Ethacrylat-Copolymer (MAE-A) den amorphen Zustand im Feststoff am besten stabilisieren. Mittels erstmalig auf Arzneistoff-Polymer-Systeme angewandter ¹H-NMR-Titration werden signifikante molekulare Wechselwirkungen mit den schwachen Basen Cinnarizin und Trimethoprim nachgewiesen. Neben Säure-Base-Interaktionen beeinflussen das Schmelzverhalten, die Glasübergangstemperatur und die Schmelzviskosität der Polymere maßgeblich die Einbettung des Wirkstoffs in die Matrix. Hinsichtlich der Langzeitstabilität neigen anfänglich kristalline ASDs zur weiteren Kristallisation, während eine Rekristallisation aus vollständig amorphen, homogenen Systemen selten auftritt. Das Auflösungsverhalten folgt bei niedriger Beladung primär den Eigenschaften des Polymers, was insbesondere für MAE-A, Copovidon und Povidon bei unterschiedlichen Wirkstoffbeladungen von 10% und 50% (m/m) bestätigt wird.

Darüber hinaus spielt die Wahl der Herstellungsmethode eine entscheidende Rolle für die Stabilität und Freisetzung. Während die HME die Bildung von ASDs mit höheren Wirkstoffbeladungen und überlegener physikalischer Stabilität ermöglicht, führt die Sprühtrocknung meist zu höherer Kristallinität, generiert jedoch bei Trimethoprim-Formulierungen eine schnelle initiale Auflösung und hohe Übersättigungswerte. Dies verdeutlicht, dass der Erfolg einer Formulierung nicht allein von der Amorphizität abhängt und herkömmliche Marker wie die XRPD-Analyse nicht immer mit der Wirkstofffreisetzung korrelieren.

Computerbasiert vorhergesagte pK_a-Werte erklären das pH-abhängige Löslichkeitsverhalten und identifizieren Protonierungsstellen. Molecular dynamics-Simulationen zeigen detailliert auf, dass bei unpolaren Systemen primär der Polymergehalt die API-Interaktionen stört, während bei polaren APIs die funktionellen Gruppen des Polymers entscheidend sind. Zusammengenommen liefert diese Studie ein detailliertes Bild der vielfältigen Faktoren, die die ASD-Performance beeinflussen. Die Kombination experimenteller und modellierender Ansätze verdeutlicht, wie Arzneistoff-Polymer-Wechselwirkungen, physikochemische Eigenschaften und Prozessparameter gemeinsam die Stabilität und das Auflösungsverhalten prägen.

7 References

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8 Appendix

8.1 Figures

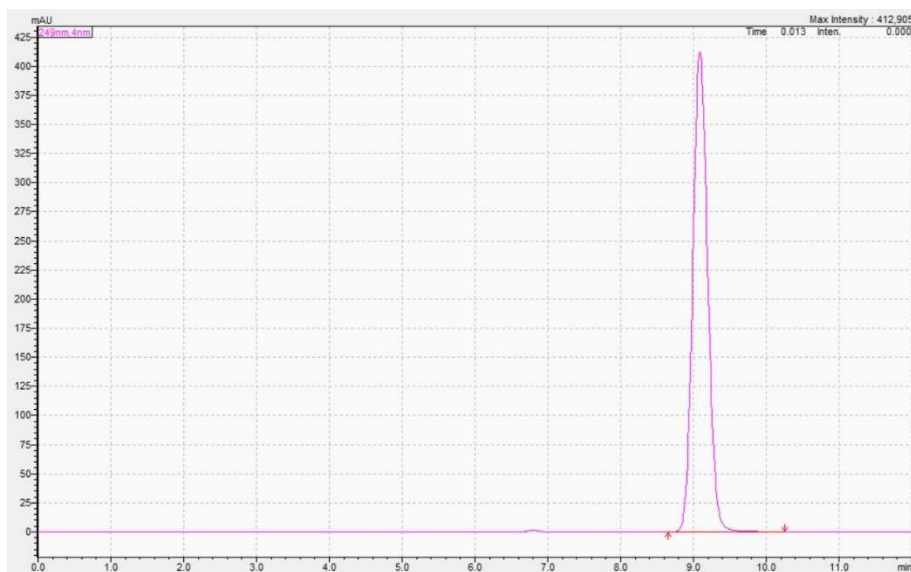


Figure 8.1 Chromatogram of cinnarizine.

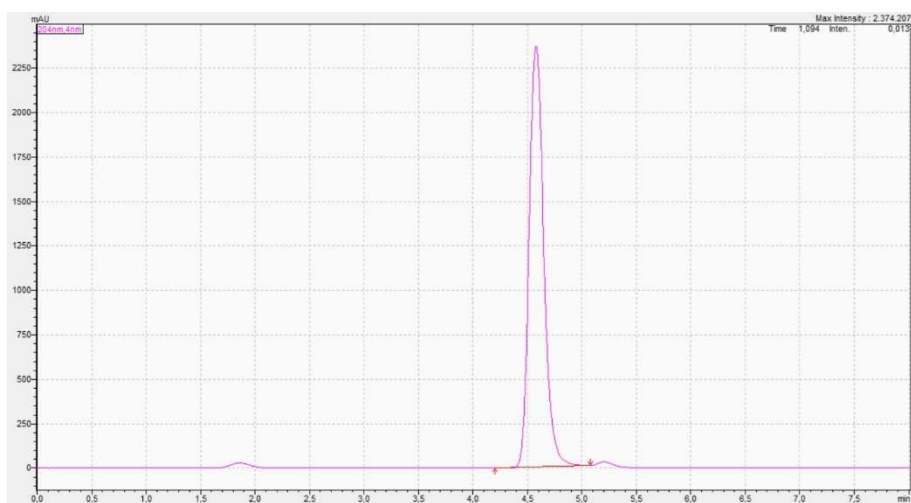


Figure 8.2 Chromatogram of trimethoprim.

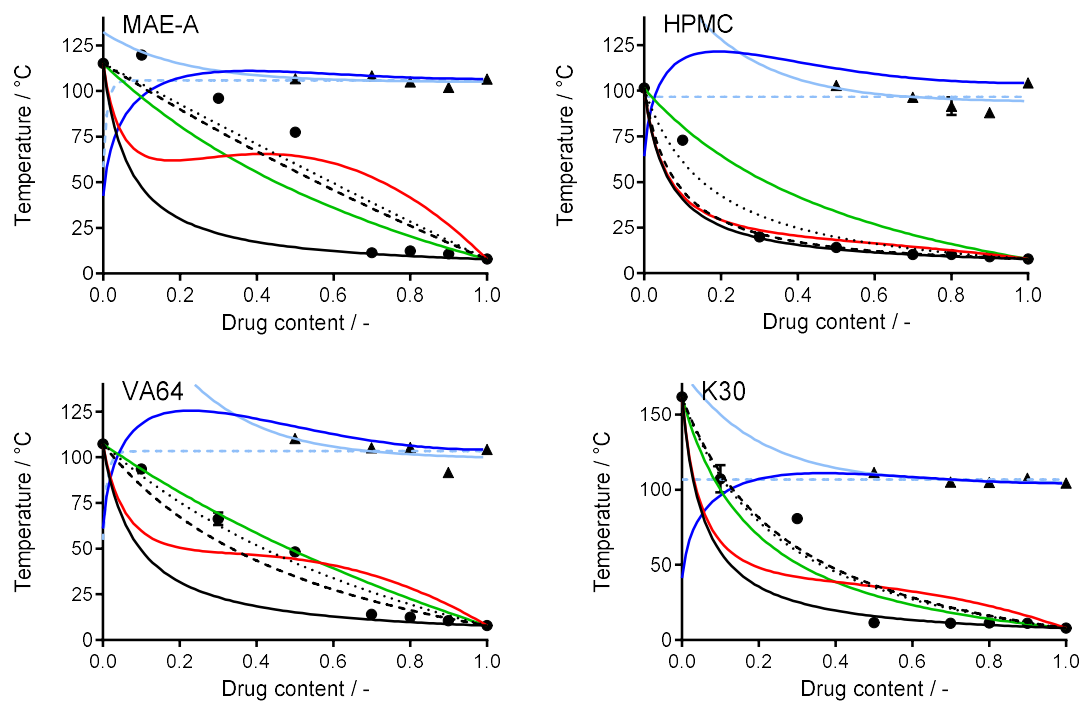


Figure 8.3 Phase diagrams of cinnarizine-MAE-A, cinnarizine-HPMC, cinnarizine-VA64 and cinnarizine-K30 amorphous solid dispersions. Circles and triangles show the measured glass transition temperatures and solubility temperatures, respectively (mean $\pm$ standard deviation, $n = 3$). The lines represent the calculated curves for Gordon-Taylor (black; solid for theoretical k , dashed for calculated k , dotted for fitted k), Kwei (red), Couchman-Karas (green), Flory-Huggins (dark blue) and Kyeremateng (light blue; solid for fixed $b = 0.05$, dashed for variable b).

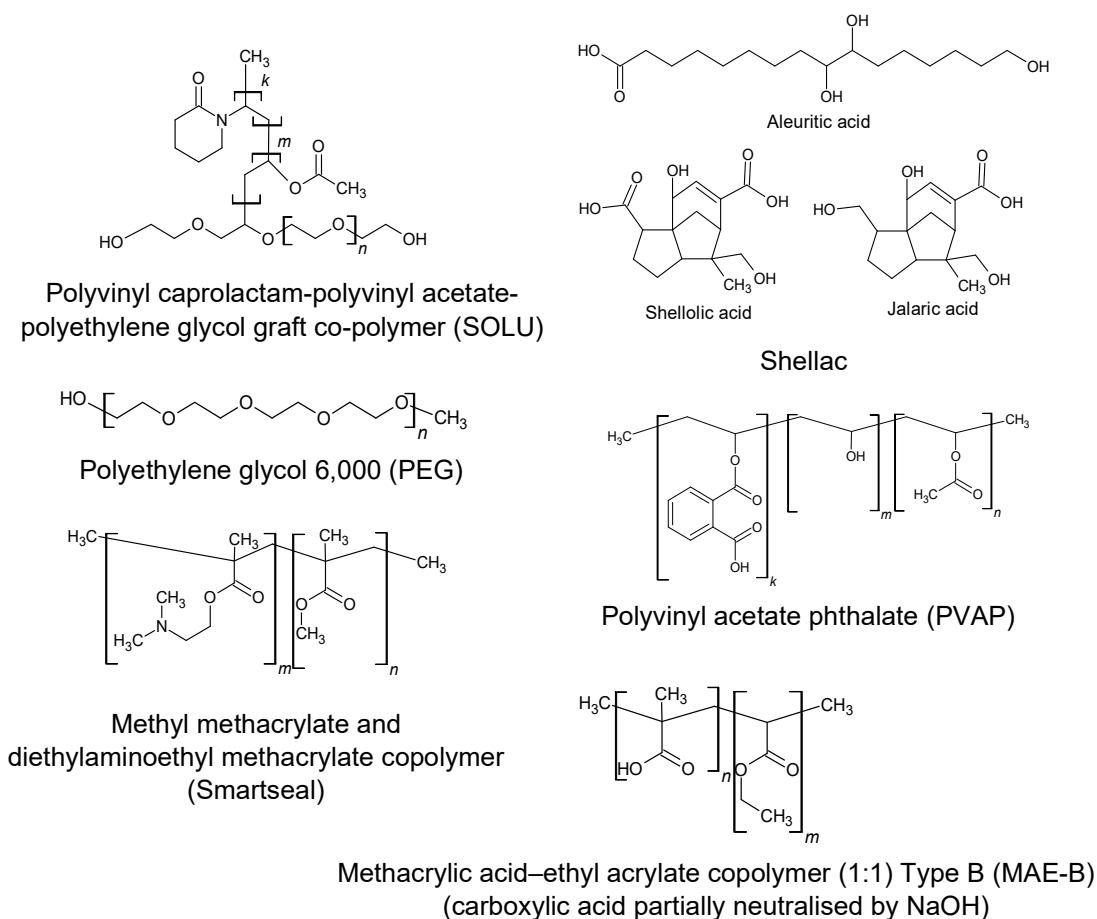


Figure 8.4 Chemical structures of additional polymers used in this work. The respective abbreviations are given in brackets.

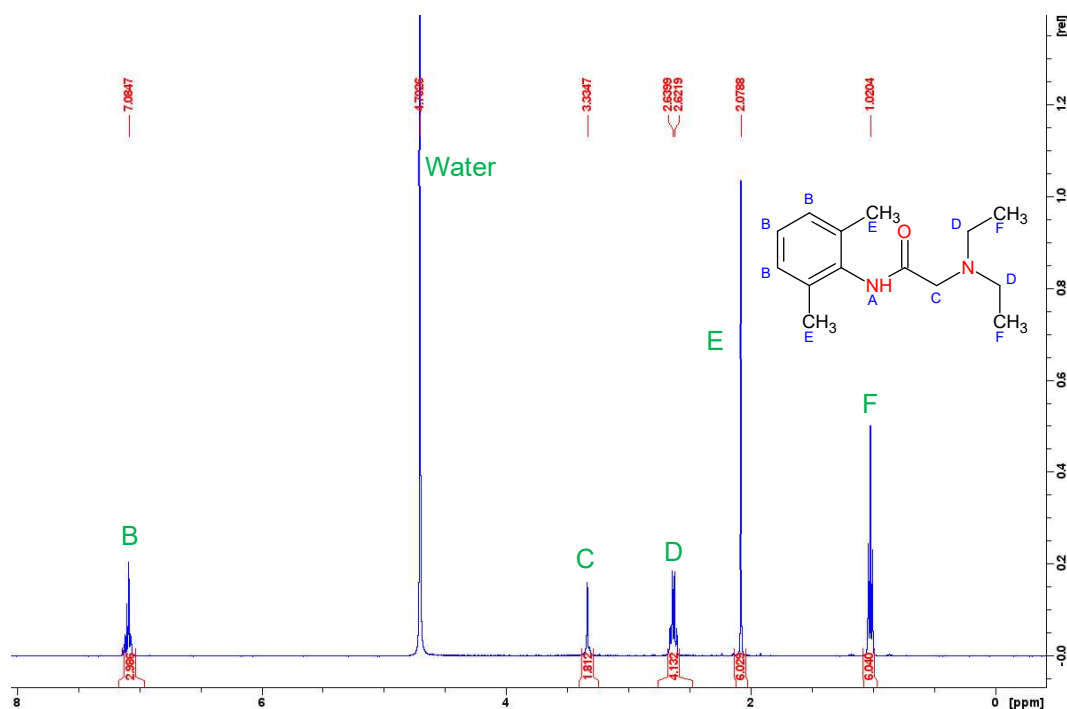


Figure 8.5 ¹H-NMR of lidocaine in deuterium oxide. Green letters show the position of protons within the molecule. Please note that position A (nitrogen-bound proton) cannot be detected in deuterium oxide.

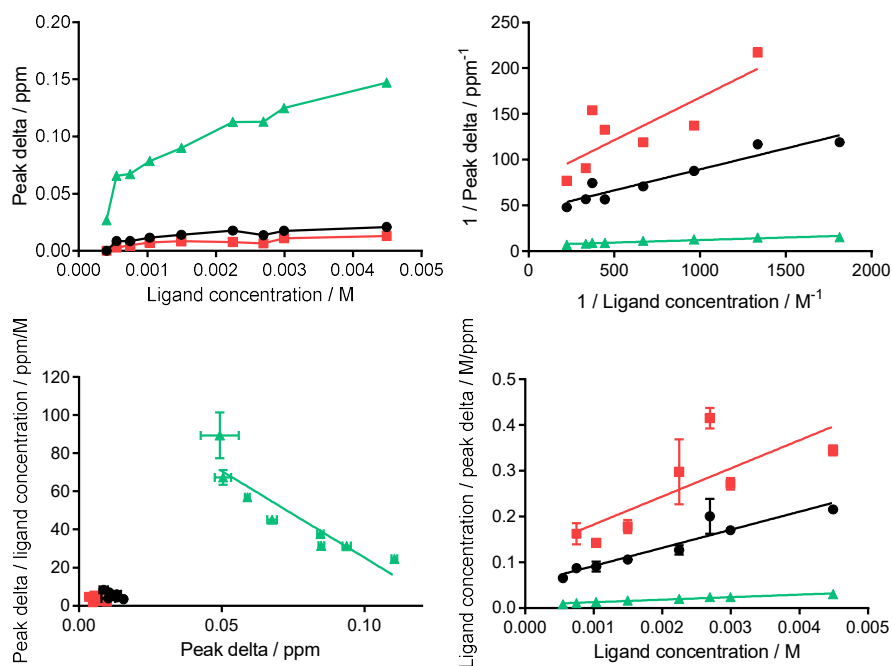


Figure 8.6 Binding curve of the lidocaine - BSA titration in deuterium oxide (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are B (black circles), E (red squares) and F (green triangles).

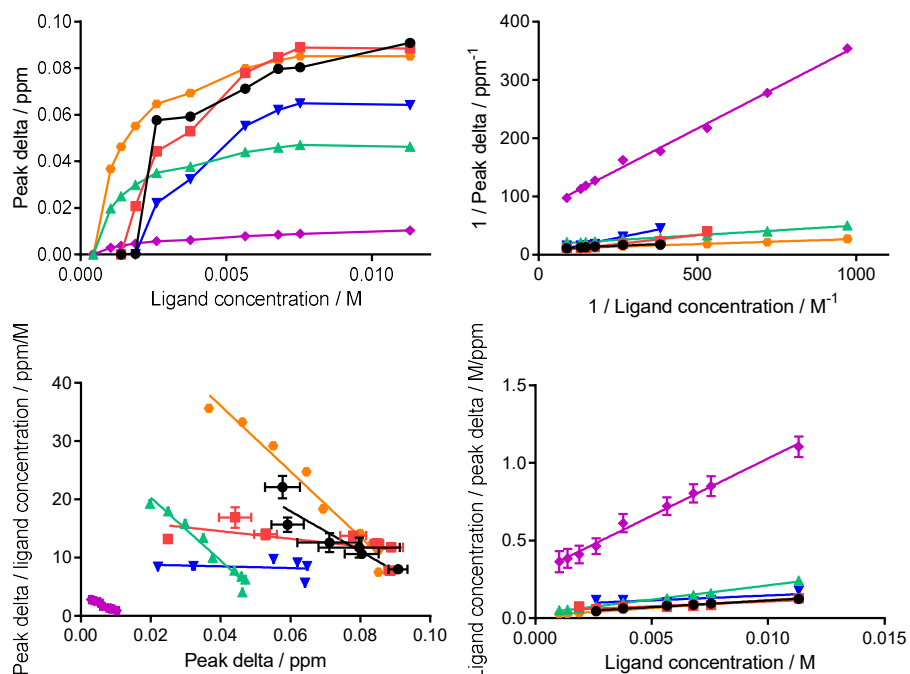


Figure 8.7 Binding curve of the cinnarizine - HPMCAS titration in methanol-d4 (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

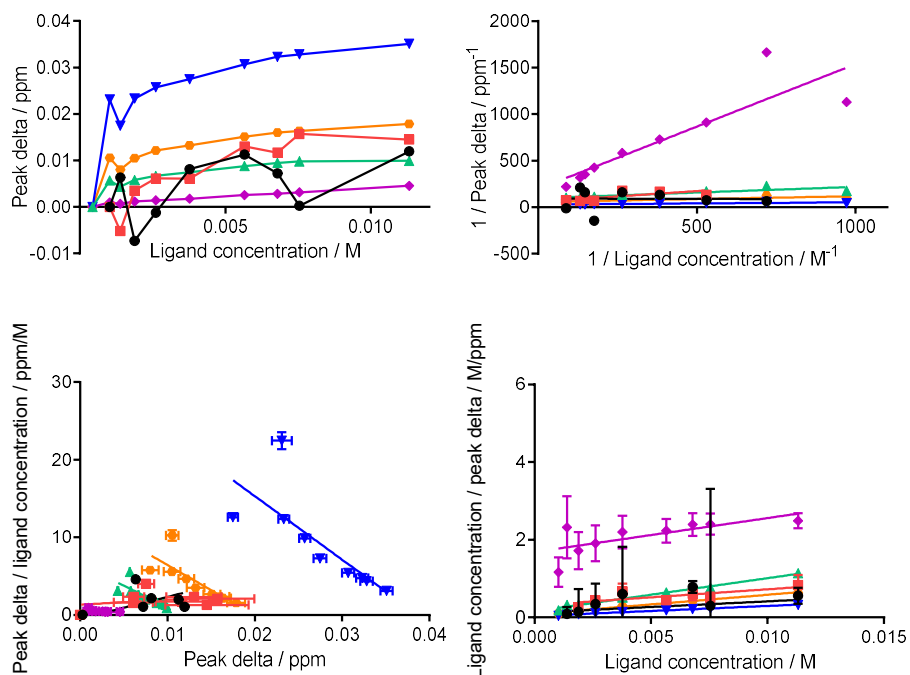


Figure 8.8 Binding curve of the cinnarizine - HPMC titration in methanol-d₄ (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

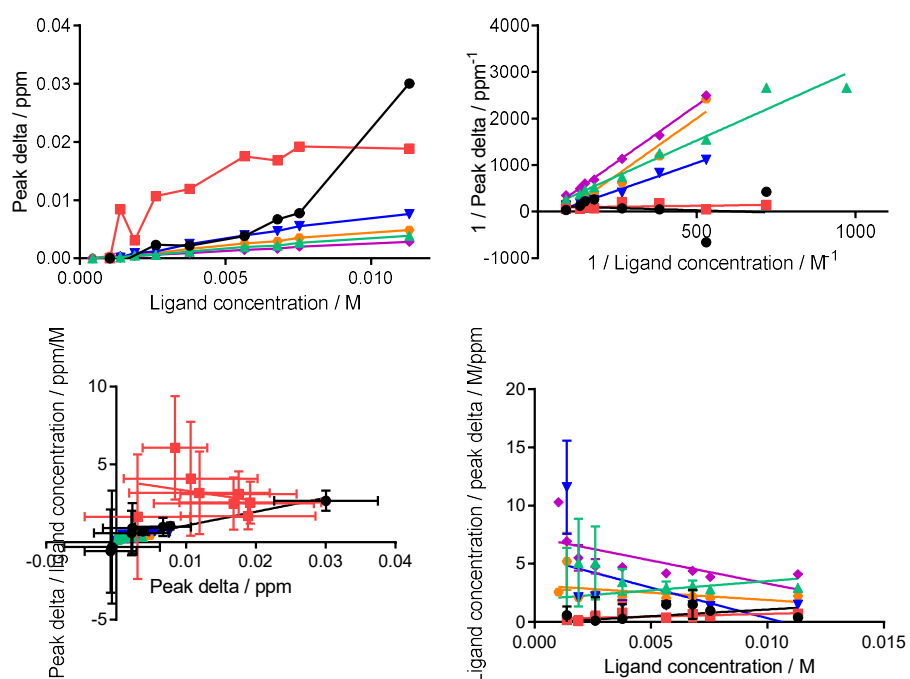


Figure 8.9 Binding curve of the cinnarizine - EPO titration in methanol-d₄ (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

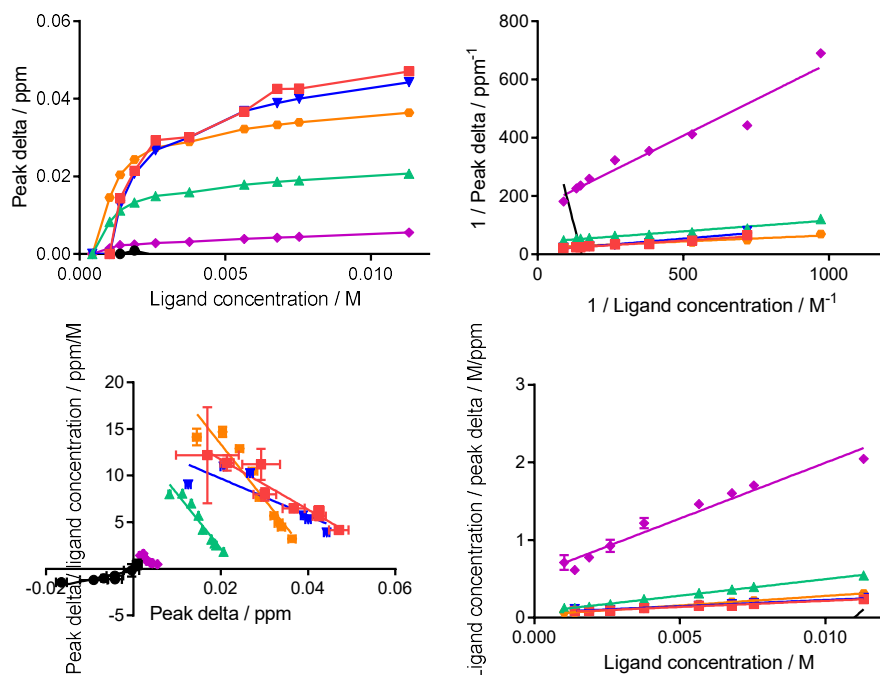


Figure 8.10 Binding curve of the cinnarizine – VA64 titration in methanol-d4 (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

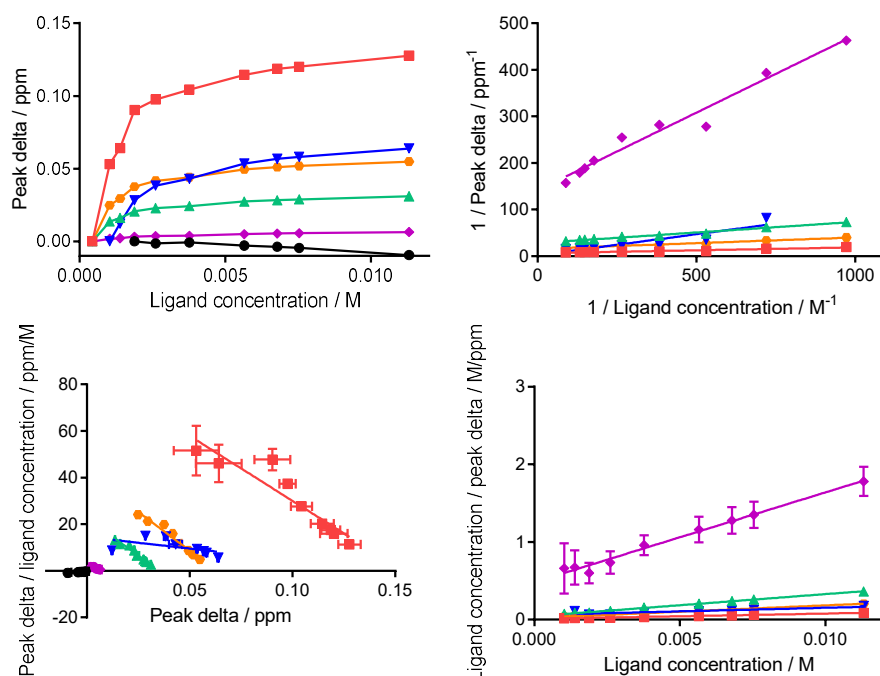


Figure 8.11 Binding curve of the cinnarizine – K30 titration in methanol-d4 (top left), Benesi-Hildebrand-plot (top right), Scatchard-plot (bottom left) and Scott-plot (bottom right). Displayed are the mean $\pm$ standard deviation ($n = 3$; small error bars are partly covered by symbols). The proton positions are F (black circles), E (red squares), C (green triangles), D (blue inverted triangles), B (purple rhombuses) and A (orange hexagons).

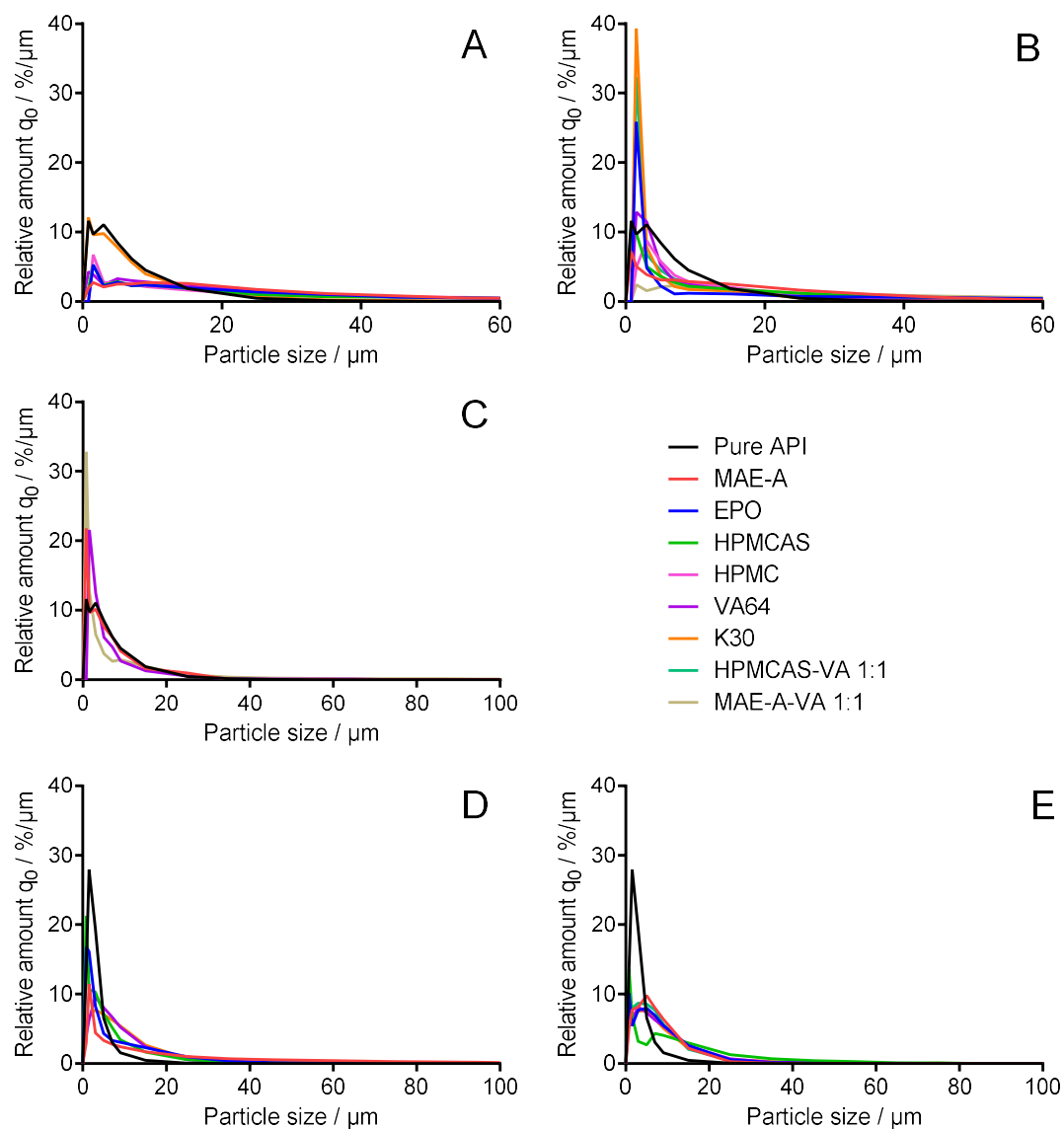


Figure 8.12 Particle size distribution (q_0) of cinnarizine and trimethoprim amorphous solid dispersions. A: Cinnarizine milled extrudates with 10% drug load. B: Cinnarizine milled extrudates with 50% drug load. C: Cinnarizine spray-dried ASDs with 50% drug load. D: Trimethoprim milled extrudates with 10% drug load. E: Trimethoprim milled extrudates with 50% drug load.

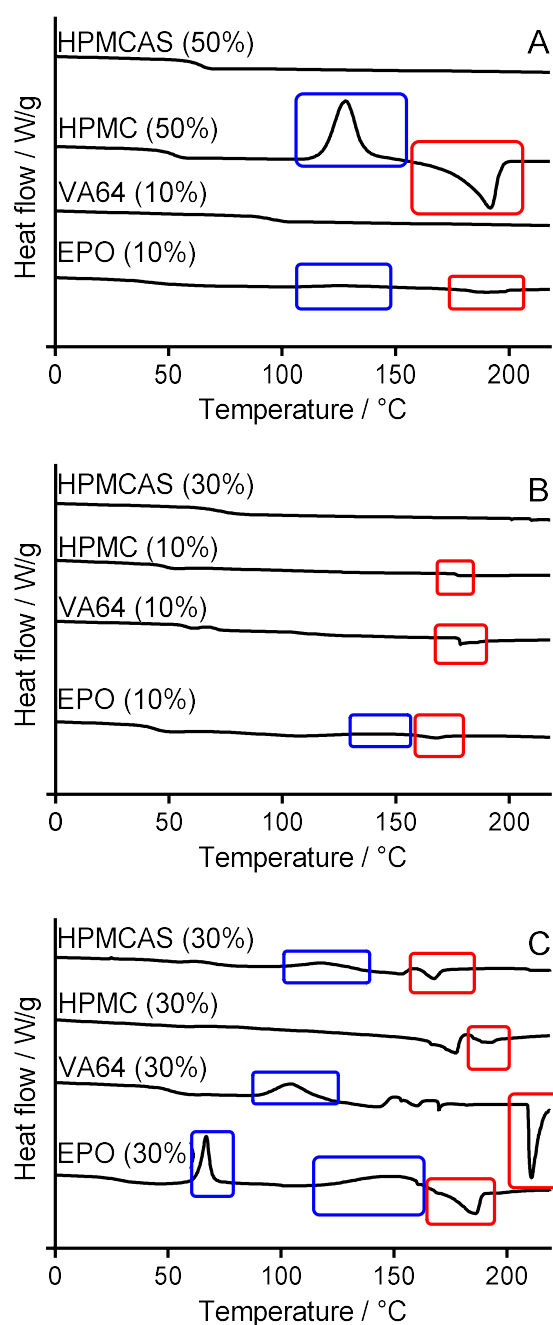


Figure 8.13 DSC plots of trimethoprim ASDs prepared by melt quenching (A), hot melt extrusion (B), and spray drying (C). Blue rectangles show crystallisation processes, red rectangles show melting processes. In melt quenching, the crystallisation and melting enthalpies of 50%-HPMC ASDs were equal, indicating an amorphous system. Shown is the first heating cycle after preparation with exothermic events plotted upwards. Calculated crystallinities are shown in Table 4.17.

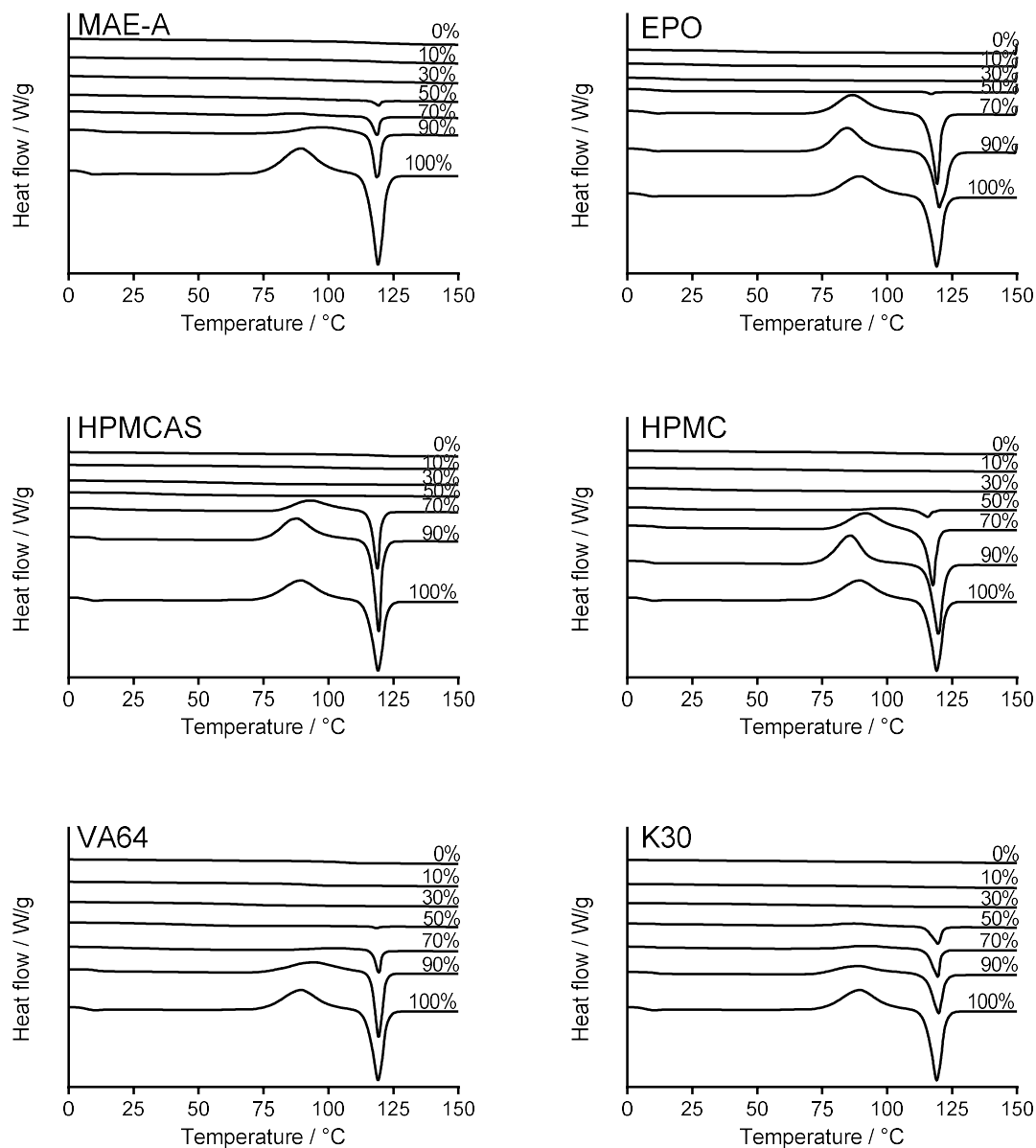


Figure 8.14 DSC plots of melt-quenched cinnarizine ASDs with MAE-A, EPO, HPMCAS, HPMC, VA64 and K30. Shown are the drug loads 0%, 10%, 30%, 50%, 70%, 90%, and 100% (w/w), respectively. Exothermal events are plotted upwards. Shown is the second heating cycle, meaning the first heating after ASD-formation.

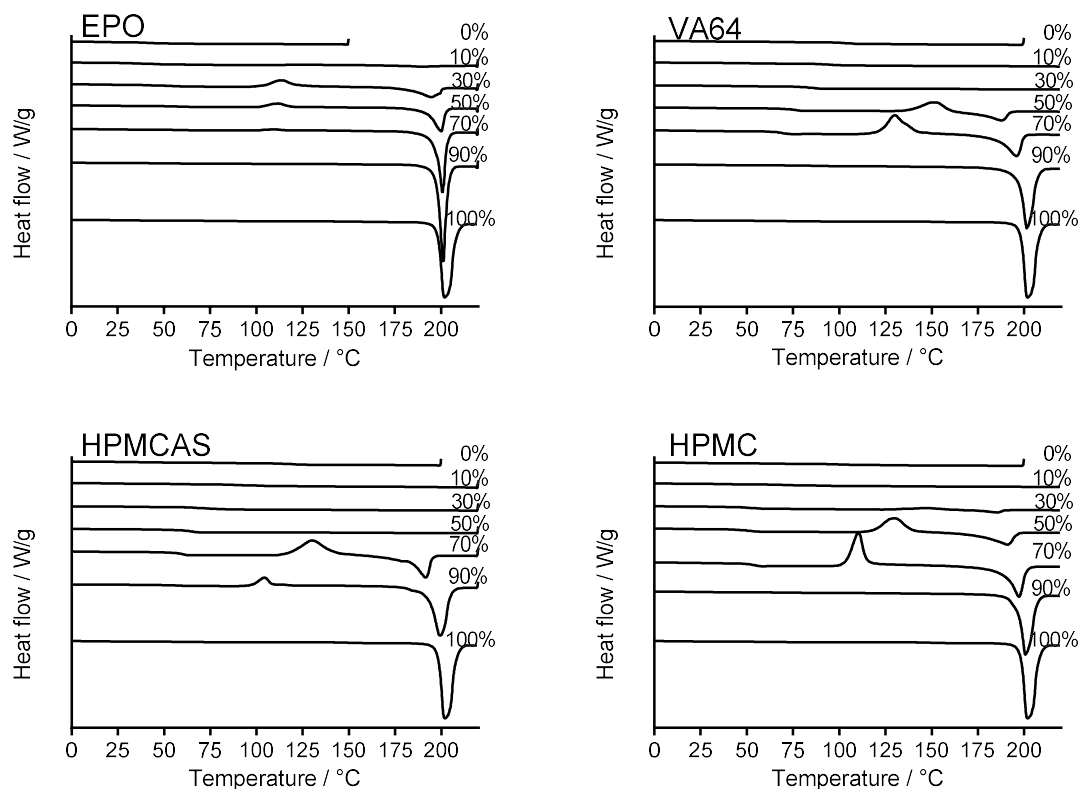


Figure 8.15 DSC plots of melt-quenched trimethoprim ASDs with EPO, HPMCAS, HPMC and VA64. Shown are the drug loads 0%, 10%, 30%, 50%, 70%, 90%, and 100% (w/w), respectively. Exothermal events are plotted upwards. Shown is the second heating cycle, meaning the first heating after ASD-formation.

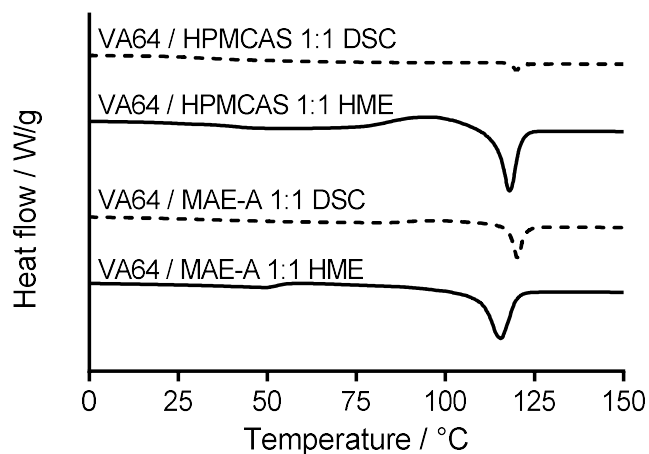


Figure 8.16 DSC plots of cinnarizine ternary ASDs with 50% (w/w) drug load. Exothermal events are plotted upwards. Shown are systems after melt quenching in the DSC (first heating cycle after ASD formation) and after hot melt extrusion (first heating cycle).

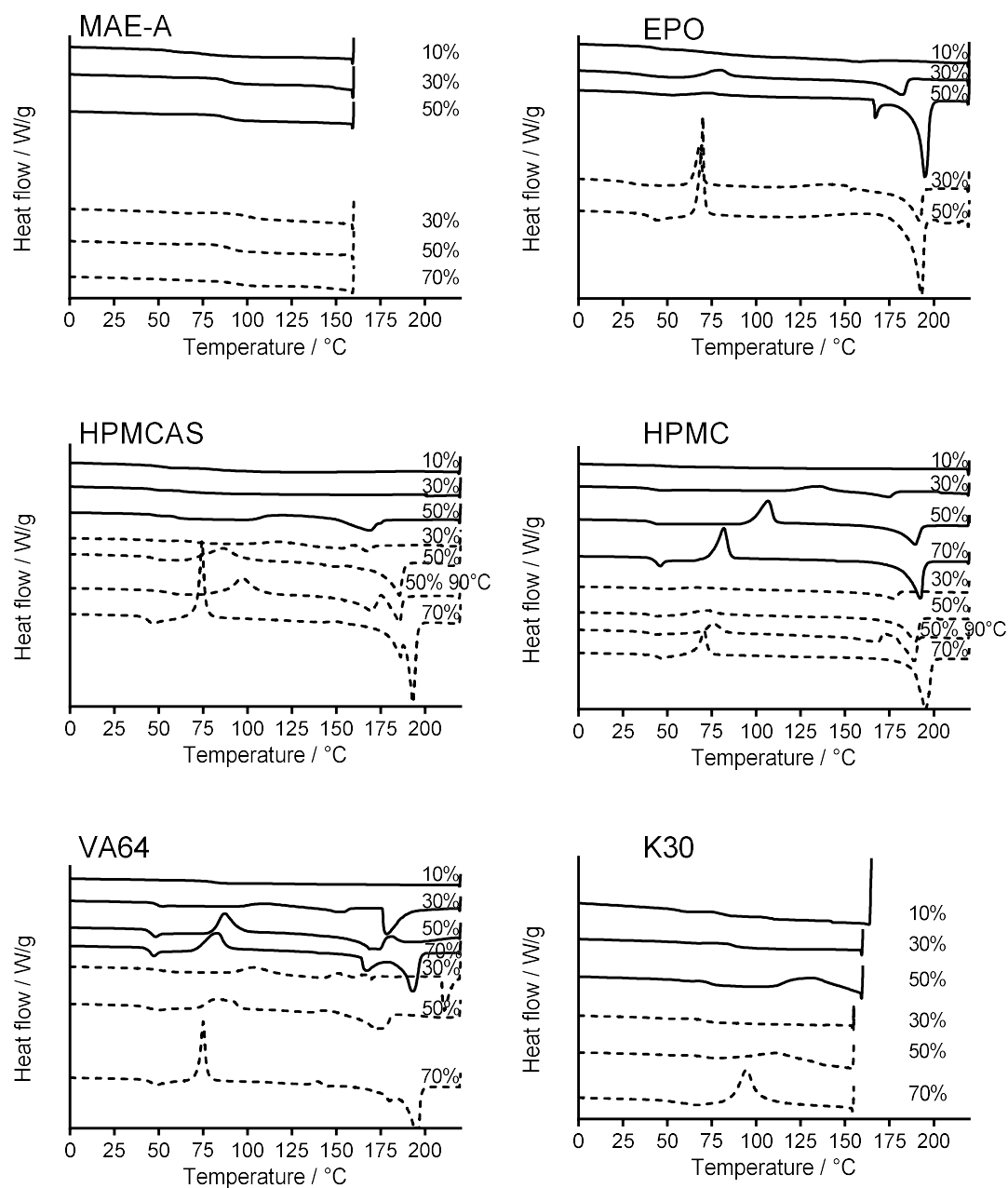


Figure 8.17 DSC plots of trimethoprim ASDs with MAE-A, EPO, HPMCAS, HPMC, VA64 and K30. Shown are ASDs prepared by HME (solid lines) and spray drying (dashed lines) at different drug loads. Exothermal events are plotted upwards. Shown is the first heating cycle.

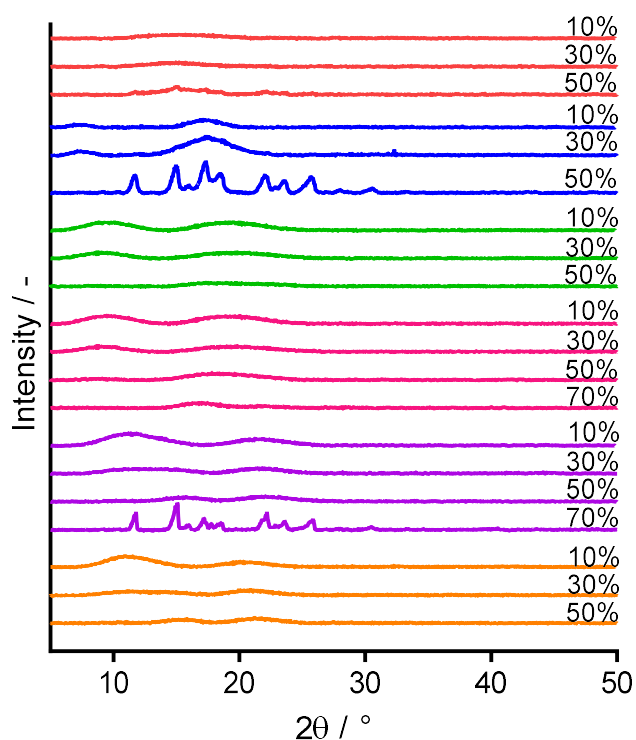


Figure 8.18 XRPD plots of trimethoprim ASDs prepared by hot melt extrusion. Shown are binary systems with MAE-A (red), EPO (blue), HPMCAS (green), HPMC (pink), VA64 (purple) and K30 (orange) with the respectively labelled drug loads (w/w). Datasets are stacked on the y-axis for better visualisation.

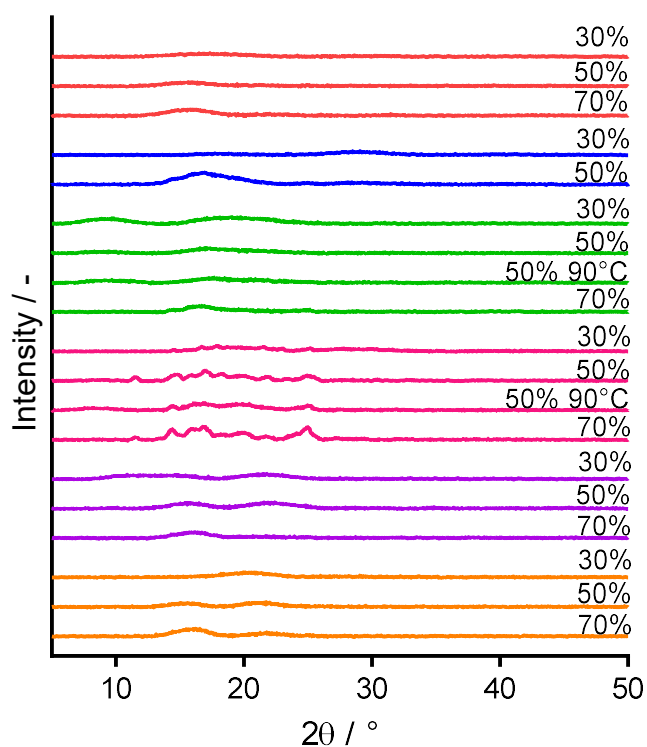


Figure 8.19 XRPD plots of trimethoprim ASDs prepared by spray drying. Shown are binary systems with MAE-A (red), EPO (blue), HPMCAS (green), HPMC (pink), VA64 (purple) and K30 (orange) with the respectively labelled drug loads (w/w). Datasets are stacked on the y-axis for better visualisation.

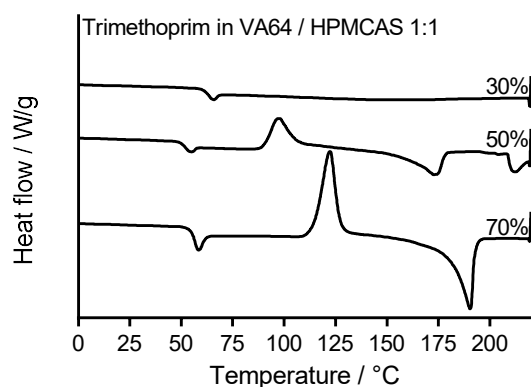


Figure 8.20 DSC plots of ternary trimethoprim ASDs in VA64 / HPMCAS (1:1) prepared by hot melt extrusion. Exothermic events are plotted upwards. Shown is the first heating cycle.

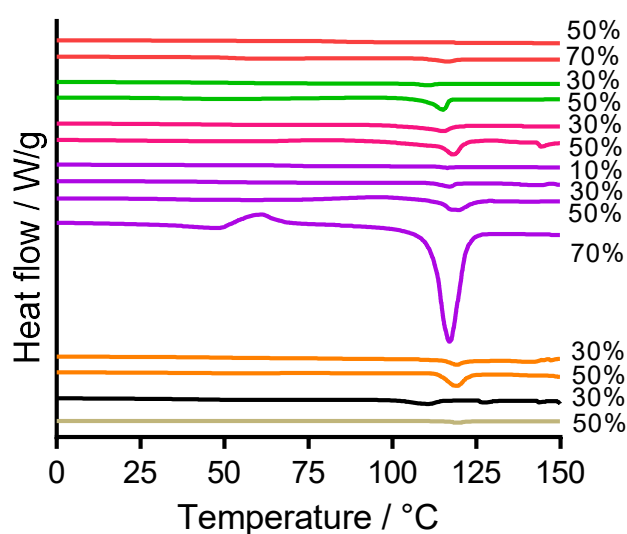


Figure 8.21 DSC plots of cinnarizine ASDs prepared by spray drying. Shown are binary systems with MAE-A (red), HPMCAS (green), HPMC (pink), VA64 (purple), K30 (orange) and Soluplus (black) with the respectively labelled drug loads (w/w). The ochre line shows the ternary system with MAE-A and VA64 (1:1). Exothermic events are plotted upwards, shown is the first heating cycle.

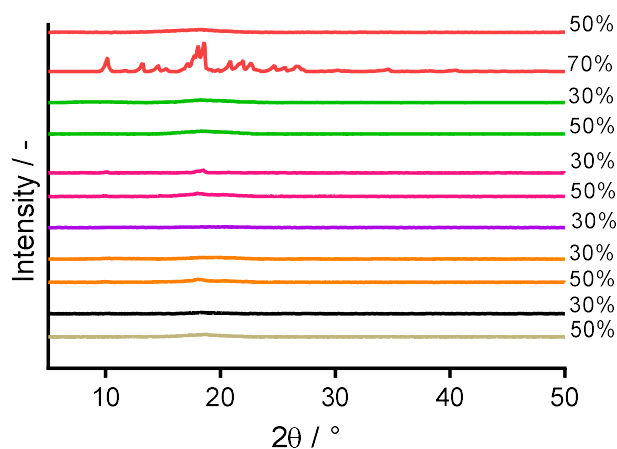


Figure 8.22 XRPD plots of cinnarizine ASDs prepared by spray drying. Shown are binary systems with MAE-A (red), HPMCAS (green), HPMC (pink), VA64 (purple), K30 (orange) and Soluplus (black) with the respectively labelled drug loads (w/w). The ochre line shows the ternary system with MAE-A and VA64 (1:1). Datasets are stacked on the y-axis for better visualisation.

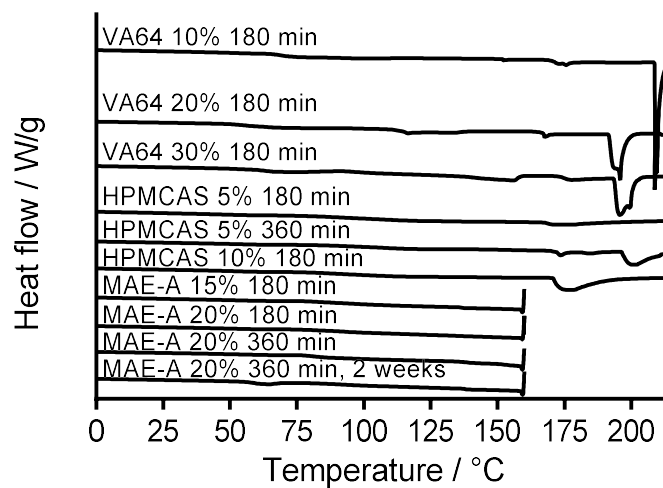


Figure 8.23 DSC plots of trimethoprim ASDs prepared by milling. Indicated are the polymer type, drug load and milling duration. The plot at the bottom shows a system after two weeks of storage under ambient conditions. Exothermal events are plotted upwards.

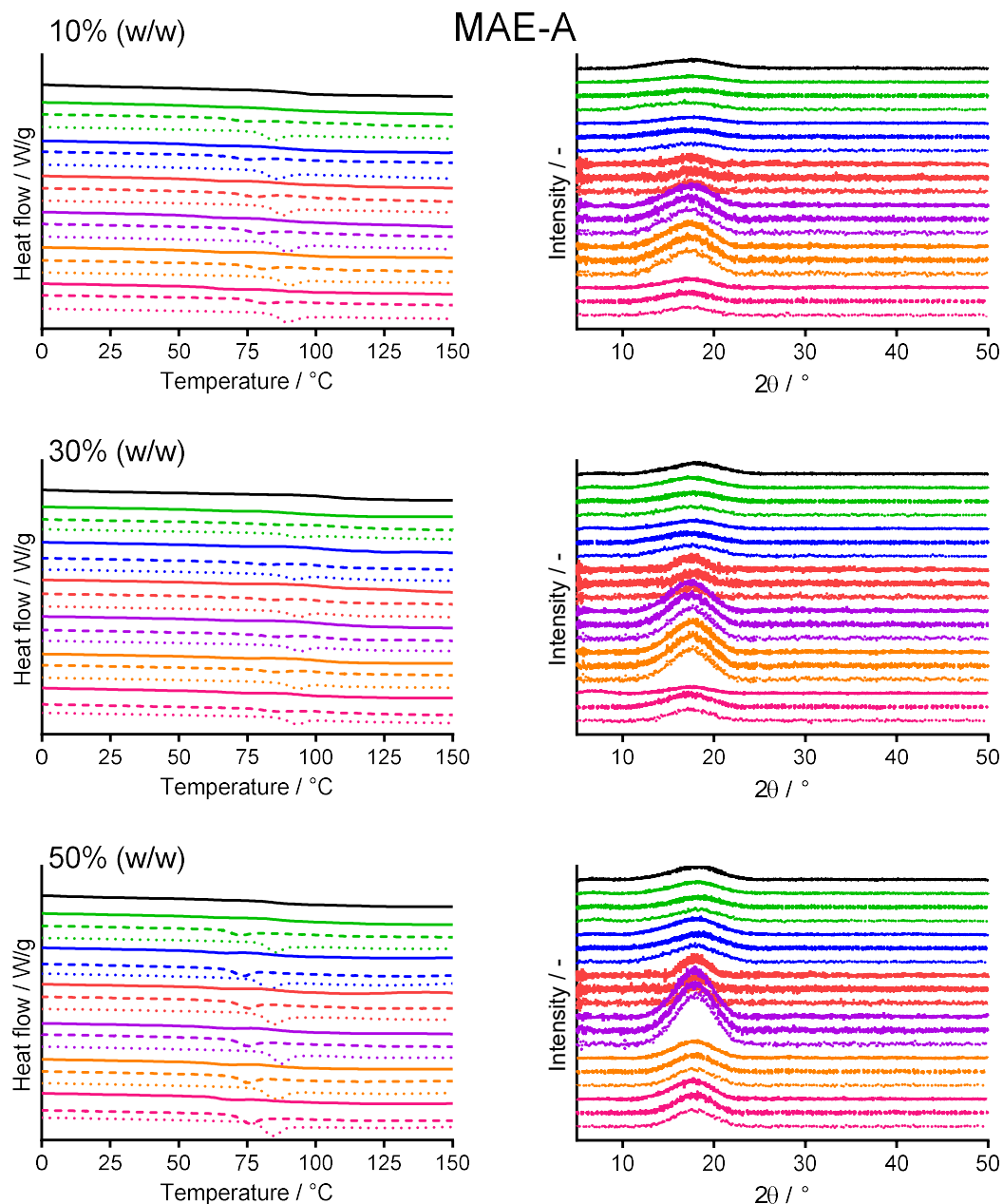


Figure 8.24 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-MAE-A-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

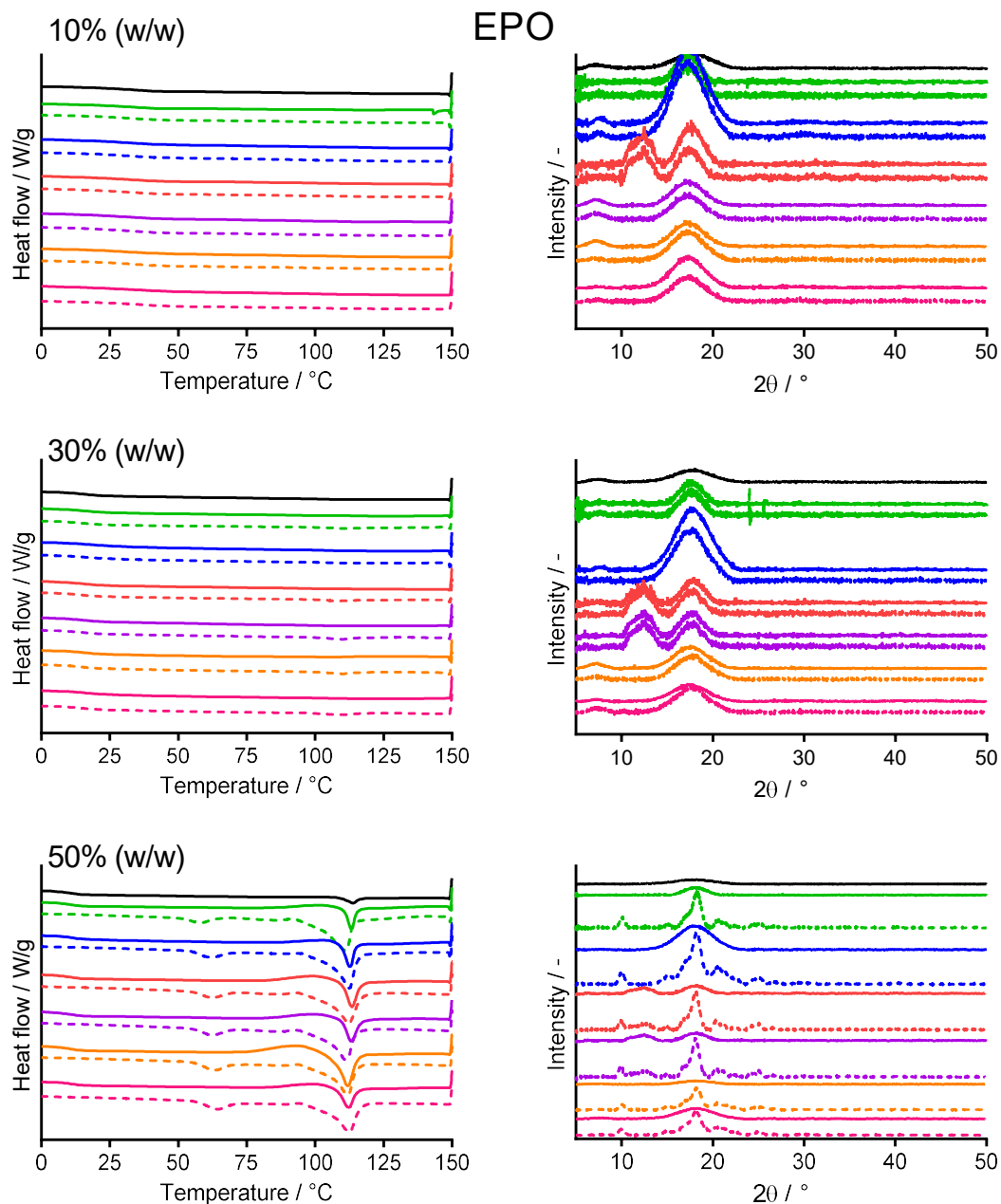


Figure 8.25 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-EPO-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line) and 25 °C / 60% rh (dashed line).

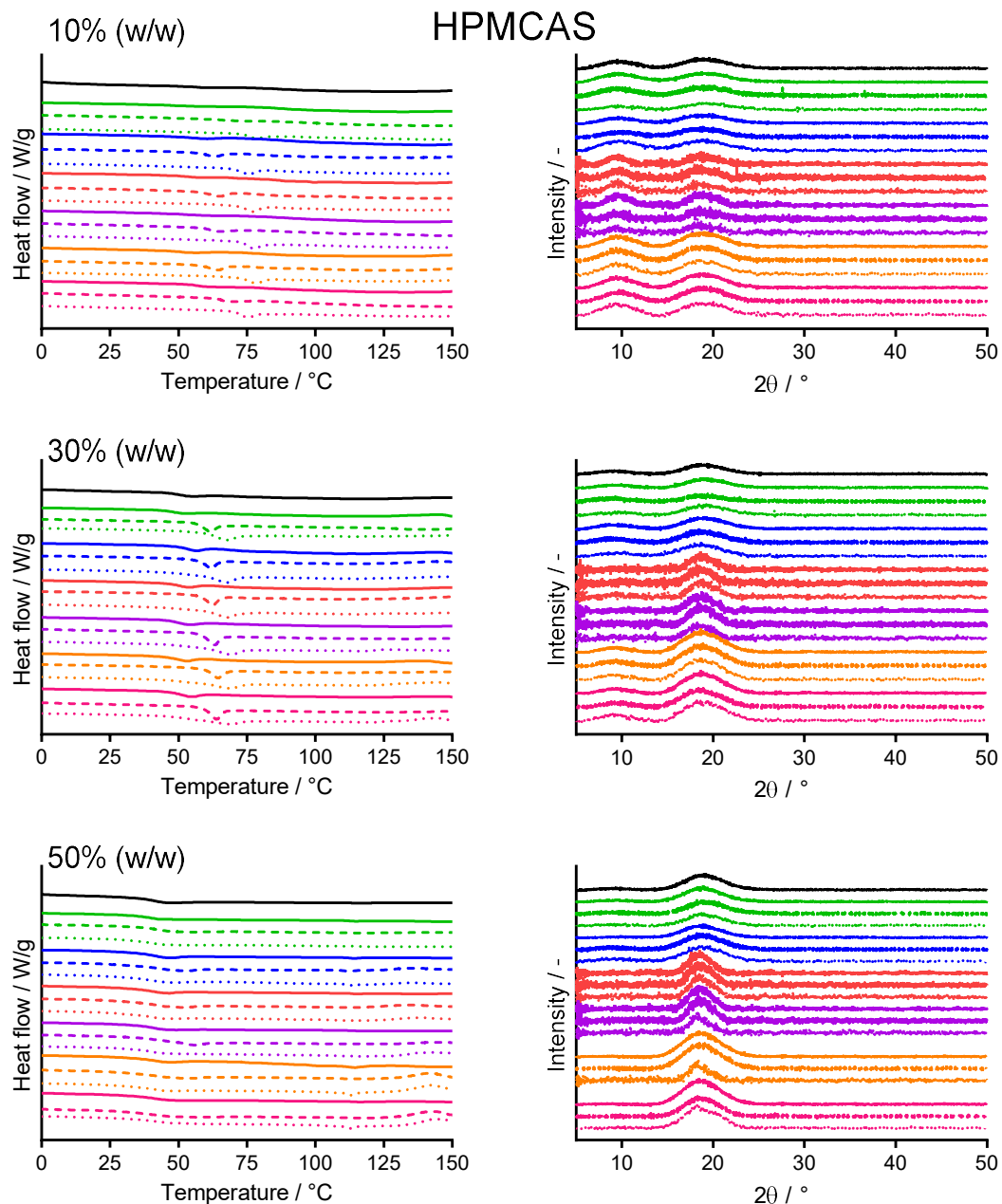


Figure 8.26 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-HPMCAS-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

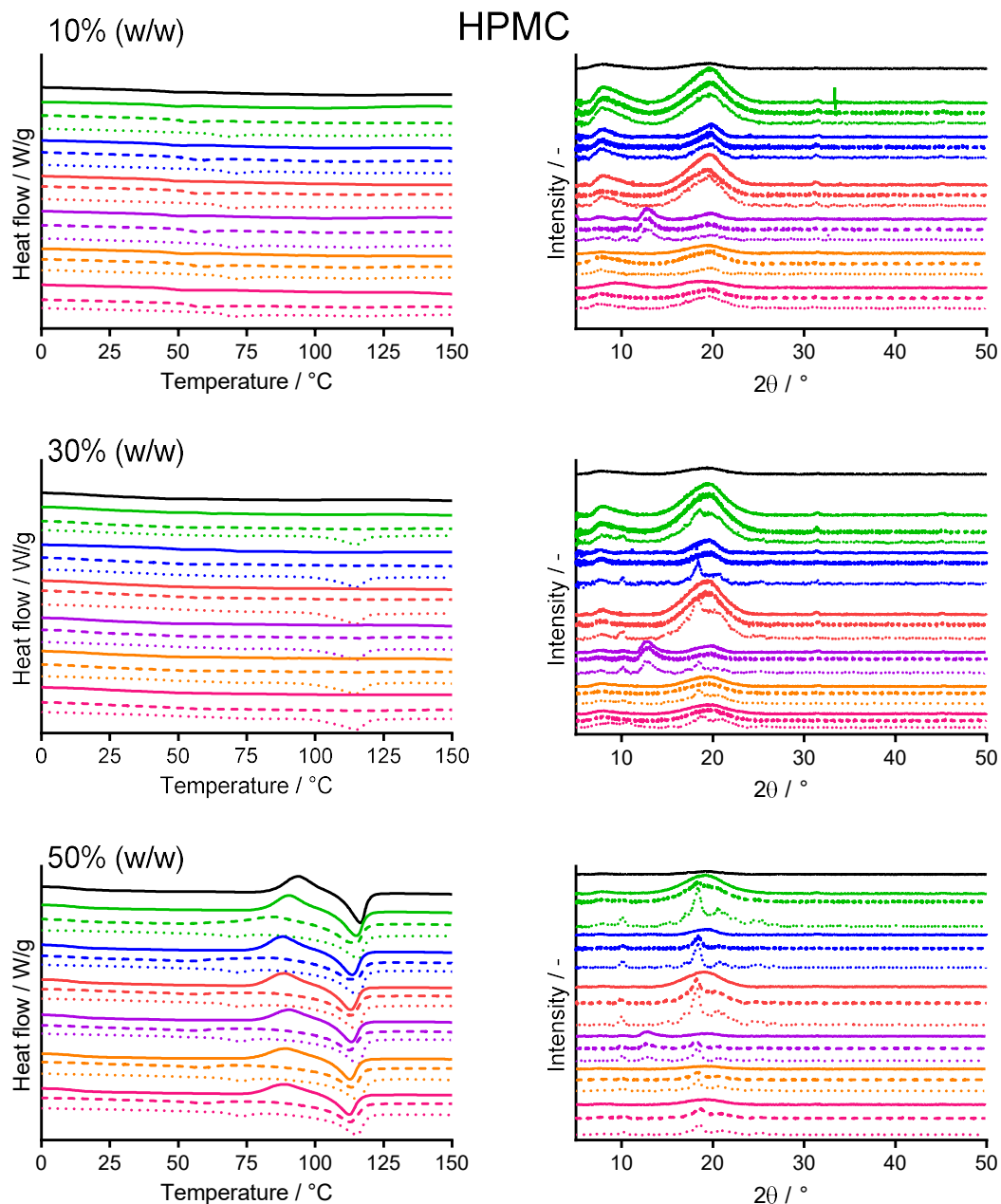


Figure 8.27 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-HPMC-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

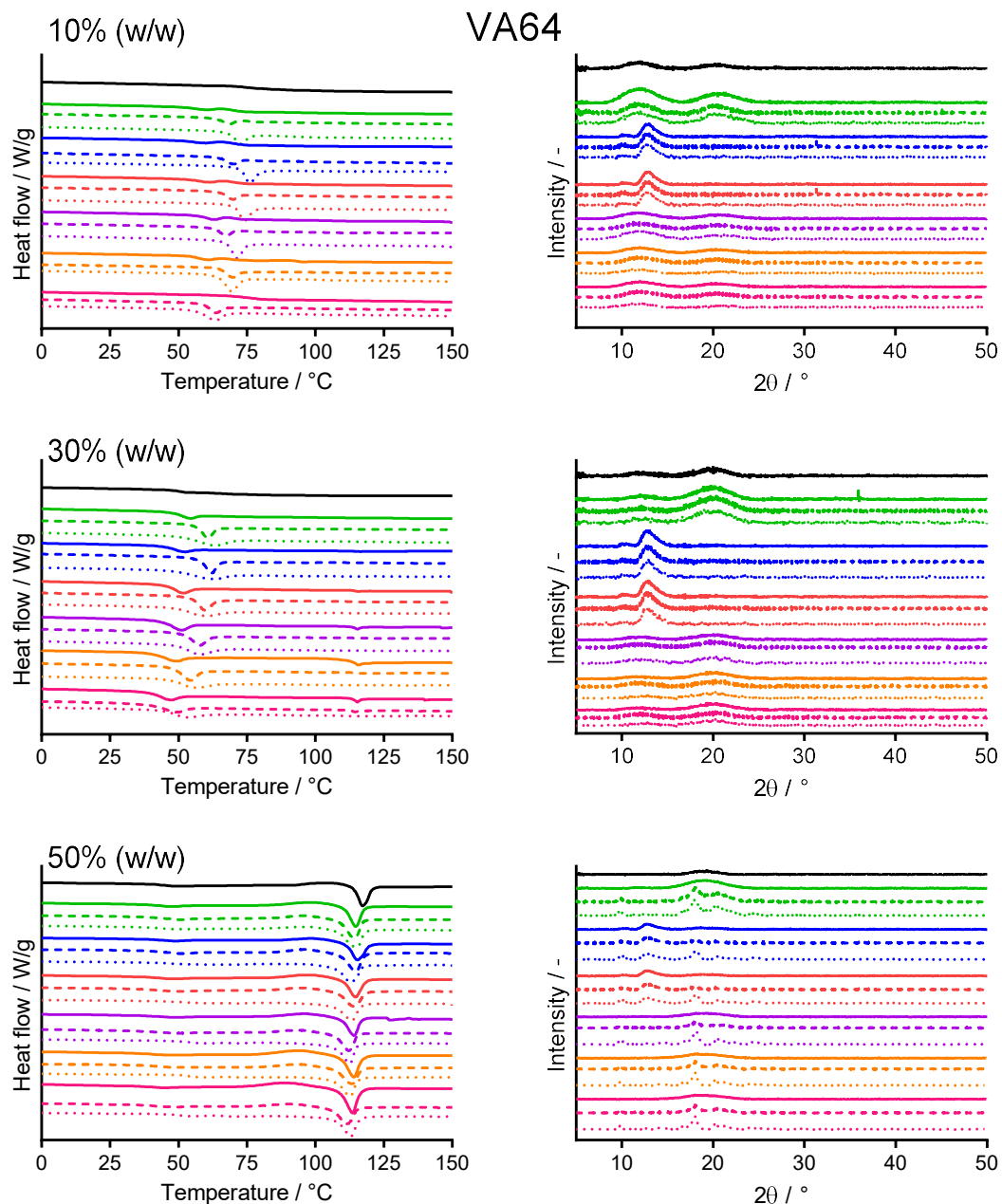


Figure 8.28 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-VA64-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

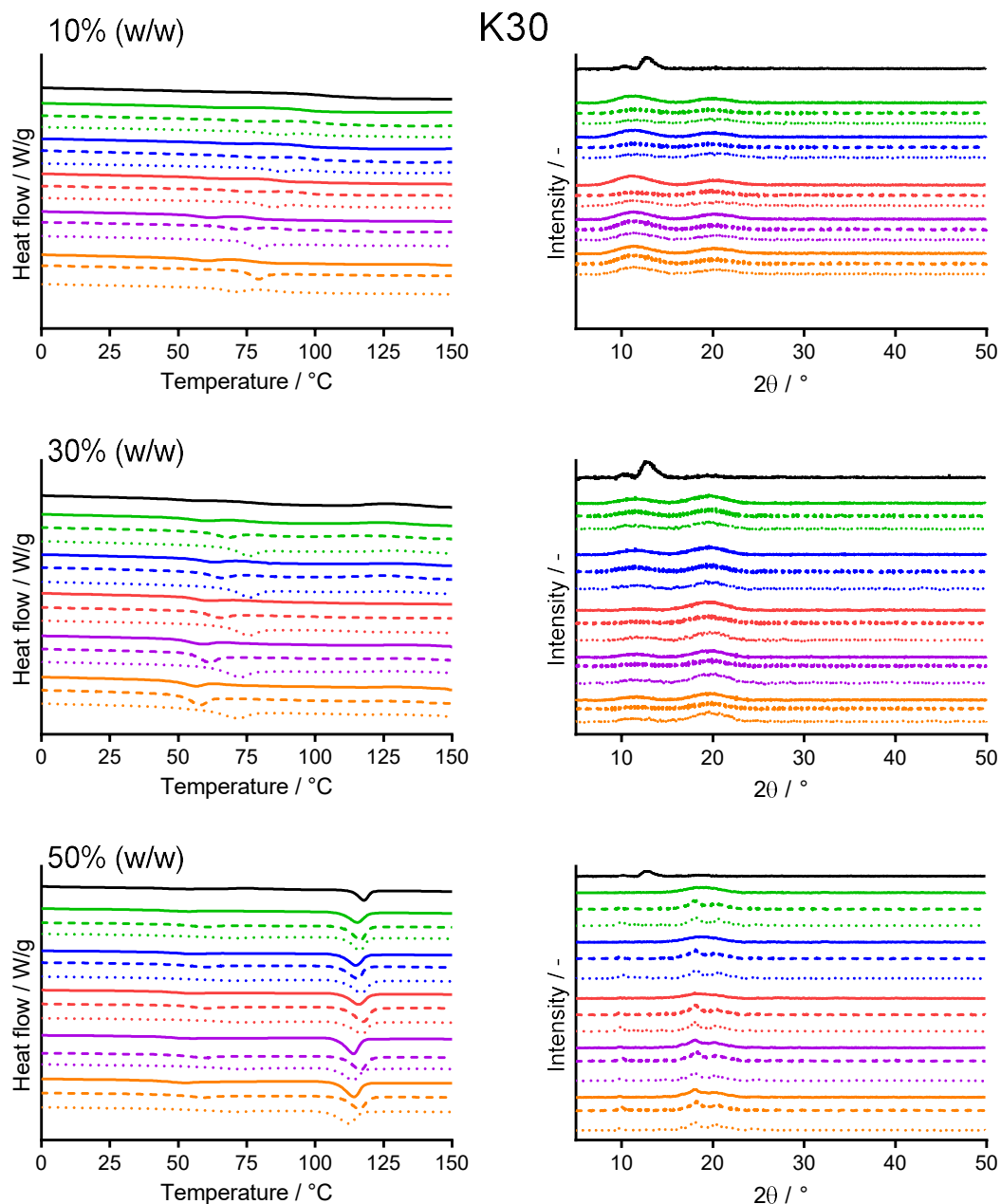


Figure 8.29 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-K30-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple) and $t = 18$ (orange) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

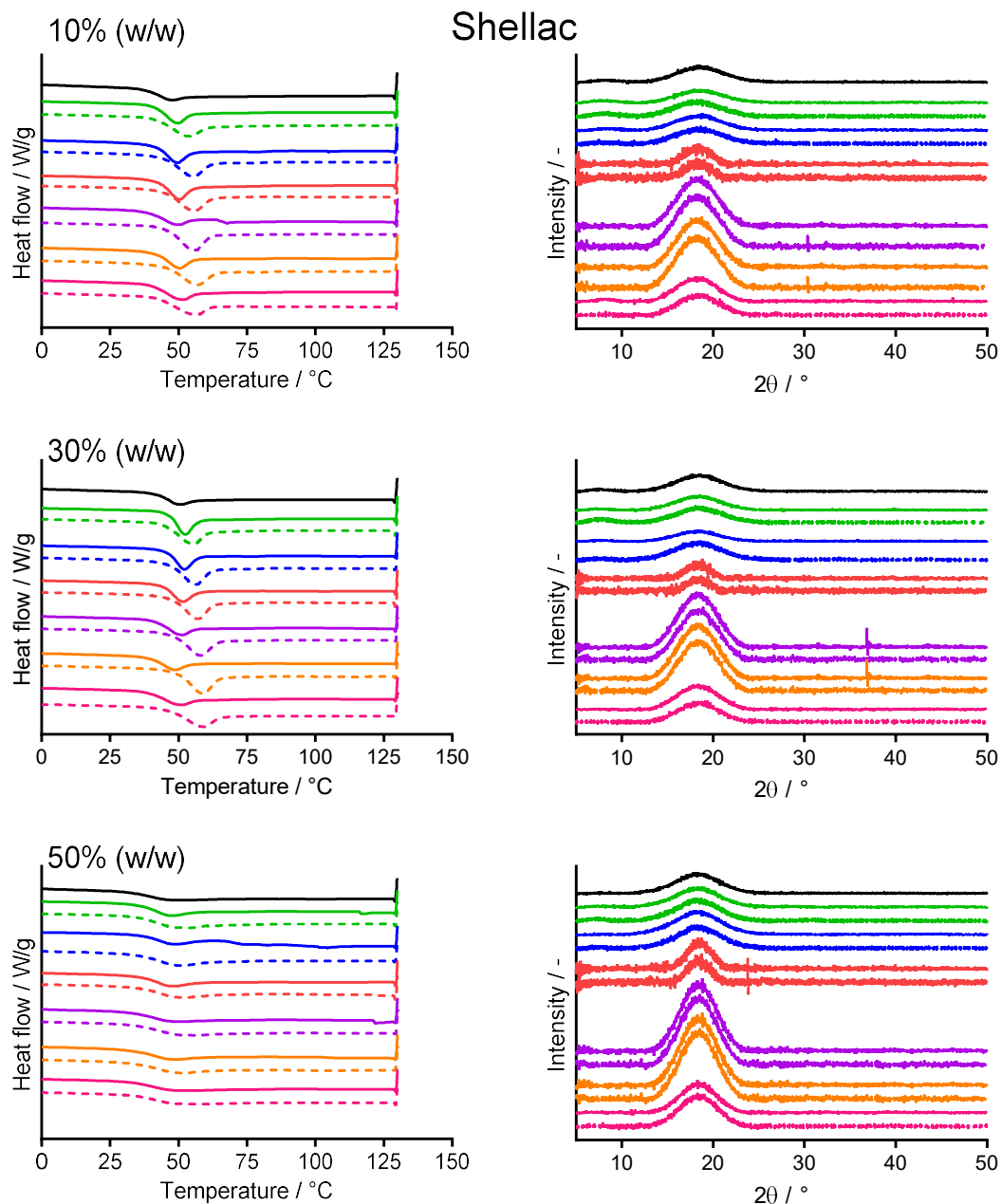


Figure 8.30 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-shellac-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are $8\text{ }^{\circ}\text{C}$ (refrigerator, solid line) and $25\text{ }^{\circ}\text{C} / 60\% \text{ rh}$ (dashed line).

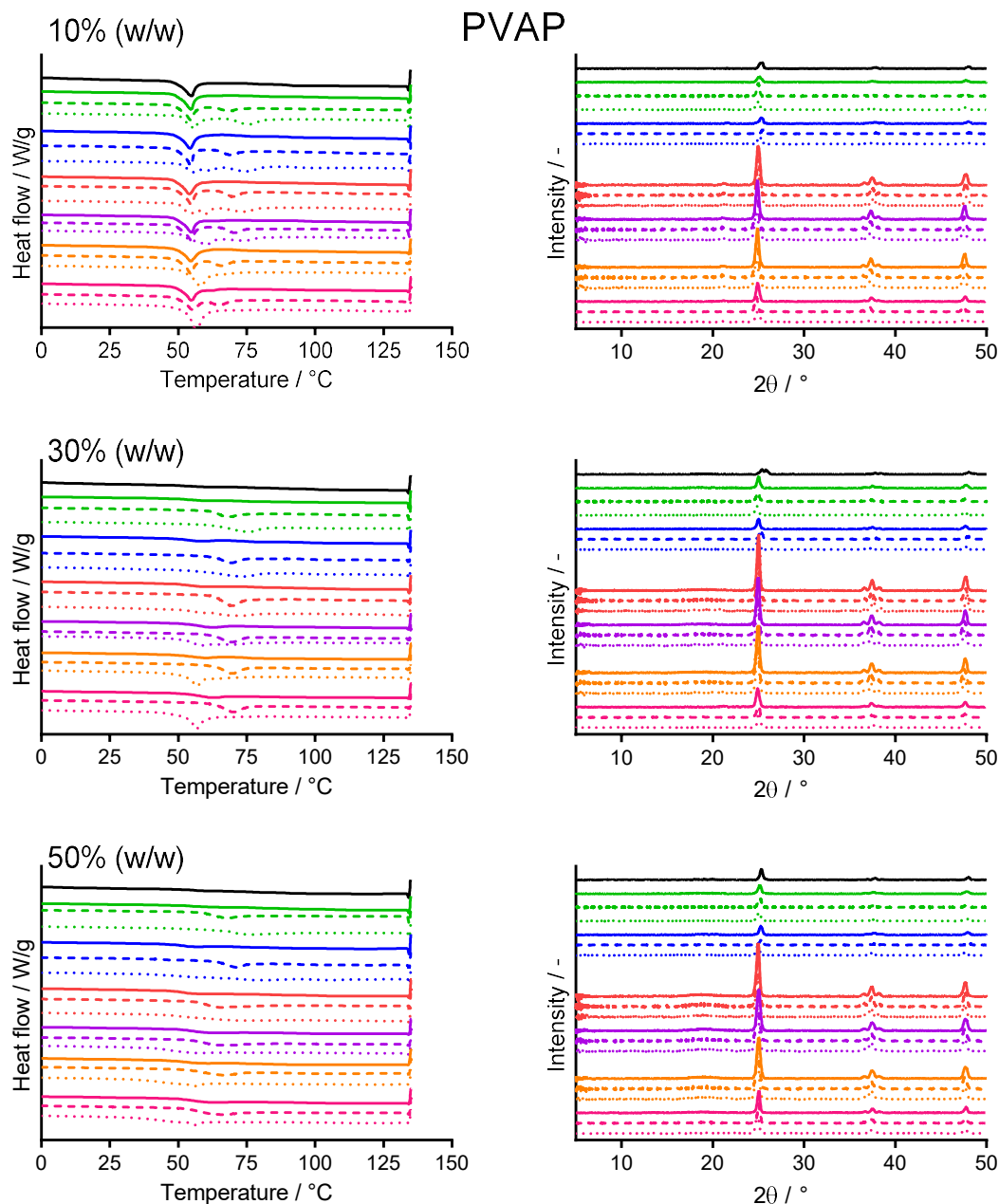


Figure 8.31 DSC-thermograms (left, exothermal events upwards) and XRPD-plots (right) of cinnarizine-PVAP-ASDs prepared by hot melt extrusion during stability testing. Shown are $t = 0$ (black), $t = 3$ (green), $t = 6$ (blue), $t = 9$ (red), $t = 12$ (purple), $t = 18$ (orange) and $t = 24$ (pink) months. Storage conditions are 8 °C (refrigerator, solid line), 25 °C / 60% rh (dashed line) and 40 °C / 75% rh (dotted line).

8.2 Tables

Table 8.1 Trimethoprim content in amorphous solid dispersions prepared by hot melt extrusion. The values represent the mean actual content $\pm$ standard deviation ($n = 3$). The relative standard deviation is given in brackets.

Polymer	Actual content			
	Target 10%	Target 30%	Target 50%	Target 70%
MAE-A	9.9 $\pm$ 0.7% (6.5%)	30.0 $\pm$ 7.0% (23.2%)	50.2 $\pm$ 0.7% (1.4%)	-/-
EPO	8.5 $\pm$ 0.3% (3.0%)	22.4 $\pm$ 1.6% (6.9%)	48.1 $\pm$ 1.9% (3.9%)	-/-
HPMCAS	7.8 $\pm$ 0.5% (6.8%)	29.3 $\pm$ 6.7% (22.7%)	50.6 $\pm$ 3.4% (6.6%)	-/-
HPMC	8.9 $\pm$ 0.6% (6.7%)	29.6 $\pm$ 0.9% (3.1%)	-/-	59.2 $\pm$ 3.6% (6.0%)
VA64	9.6 $\pm$ 0.1% (1.2%)	29.9 $\pm$ 4.2% (14.0%)	50.0 $\pm$ 6.0% (12.0%)	58.8 $\pm$ 8.5% (14.4%)
K30	10.5 $\pm$ 1.1% (10.7%)	28.8 $\pm$ 3.4% (11.7%)	53.5 $\pm$ 5.9% (11.0%)	-/-
VA64/HPMCAS 1:1	-/-	26.1 $\pm$ 1.5% (5.7%)	45.8 $\pm$ 0.4% (0.9%)	62.7 $\pm$ 6.3% (10.0%)

Table 8.2 Trimethoprim content in amorphous solid dispersions prepared by spray drying. The values represent the mean actual content $\pm$ standard deviation ($n = 3$). The relative standard deviation is given in brackets.

Polymer	Actual content			
	Target 30%	Target 50%	Target 50% (90 °C sample)	Target 70%
MAE-A	34.4 $\pm$ 0.4% (1.3%)	52.2 $\pm$ 4.1% (7.9%)	-/-	68.7 $\pm$ 0.8% (1.2%)
EPO	31.4 $\pm$ 2.3% (7.4%)	46.3 $\pm$ 4.7% (10.2%)	-/-	-/-
HPMCAS	33.4 $\pm$ 1.1% (3.2%)	52.0 $\pm$ 1.7% (3.2%)	52.7 $\pm$ 2.8% (5.3%)	71.0 $\pm$ 5.3% (7.4%)
HPMC	32.0 $\pm$ 0.3% (0.8%)	52.4 $\pm$ 2.5% (4.8%)	53.1 $\pm$ 2.4% (4.6%)	71.3 $\pm$ 2.6% (3.6%)
VA64	28.2 $\pm$ 5.3% (18.6%)	47.3 $\pm$ 0.1% (0.3%)	-/-	70.4 $\pm$ 3.5% (5.0%)
K30	30.9 $\pm$ 5.4% (17.3%)	52.1 $\pm$ 1.2% (2.3%)	-/-	72.2 $\pm$ 0.6% (0.9%)

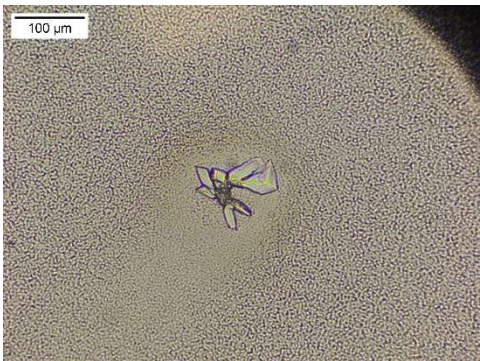
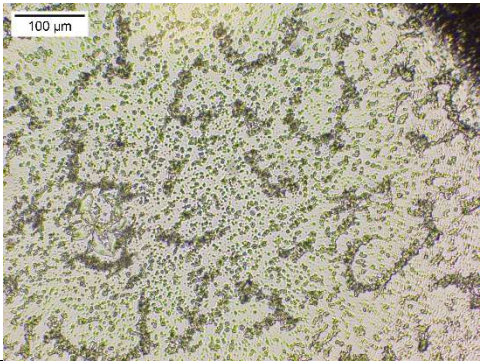
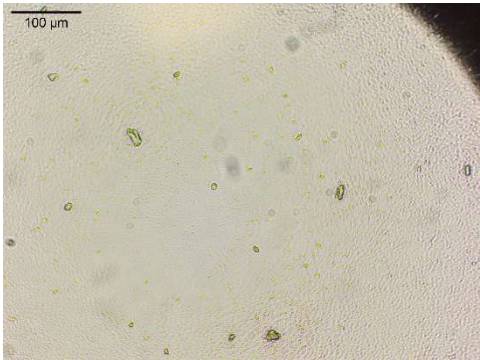
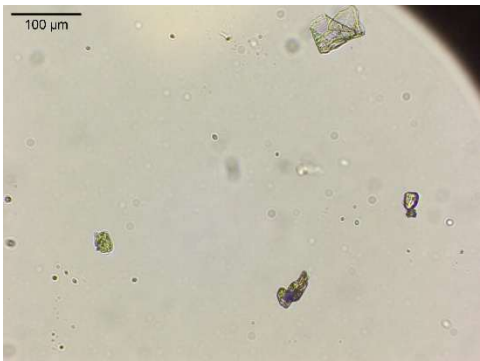
Table 8.3 Physical stability of the prepared cinnarizine amorphous solid dispersions with shellac and PVAP. The storage times are given in months. No crystallisation was found in the systems, therefore only two classifications are listed.

Polymer	Drug load [%]	Refrigerator							25 °C / 60% r.h.							40 °C / 75% r.h.						
		0	3	6	9	12	18	24	3	6	9	12	18	24	3	6	9	12	18	24		
Shellac	10																			could not be measured, as formulations melted at 40 °C		
	30																					
	50																					
PVAP	10																					
	30																					
	50																					
fully amorphous (only one T_g)									amorphous-amorphous phase separation (two or more T_{gs})													

Table 8.4 Summary of the residual crystallinity of cinnarizine amorphous solid dispersions. Numbers in red boxes indicate the degree of crystallinity relative to the whole system in percent as determined by the melting enthalpy in DSC (mean of n = 3). Green boxes indicate amorphous systems without residual crystallinity.

Polymer	Drug load / %	Hot melt extrusion	Spray drying	Melt quenching
MAE-A	10		-/-	
	30		-/-	
	50		1.2	2.2
	70	-/-	6.2	5.0
EPO	10		-/-	
	30		-/-	
	50	1.8	-/-	0.5
HPMCAS	10		-/-	
	30		4.3	
	50		8.7	
HPMC	10		-/-	
	30		18.9	
	50	1.5	24.1	1.3
VA64	10		2.1	
	30		11.8	
	50	12.3	6.8	0.7
	70	-/-	22.6	4.6
K30	10		-/-	
	30		9.5	
	50	7.6	27.4	7.2
HPMCAS / VA64 1:1	50	6.9	-/-	2.1
MAE-A / VA64 1:1	50	20.5	2.4	5.1

Table 8.5 Trimethoprim crystals formed during film casting at the specified drug load (w/w) after drying in the vacuum drying cabinet.

MAE-A	70%	
EPO	30%	
HPMCAS	70%	
HPMC	30%	
	50%	

VA64

30%



K30

30%

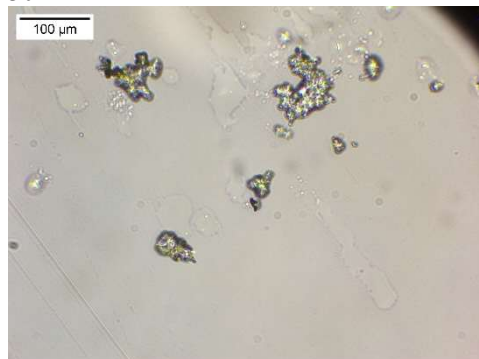
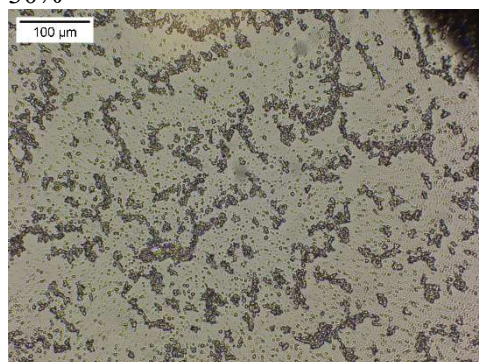


Table 8.6 Trimethoprim crystals formed during film casting at the specified drug load (w/w) after drying in the laminar airflow box.

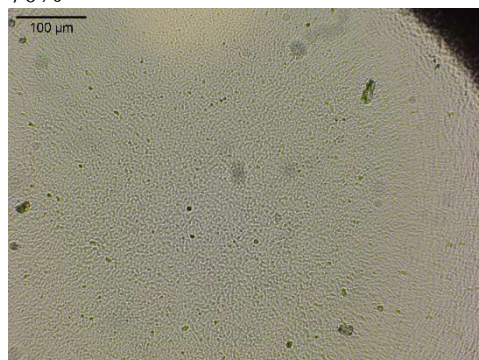
EPO

30%



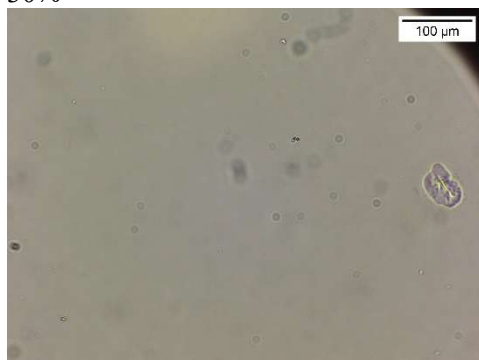
HPMCAS

70%



HPMC

30%



70%



VA64

30%



K30

30%



50%



Danksagung

Die vorliegende Dissertation entstand unter gemeinsamer Betreuung der Heinrich-Heine-Universität Düsseldorf und der Technischen Hochschule Köln.

Seitens der HHU gilt mein besonderer Dank meinem Doktorvater Herr Prof. Dr. Breitkreutz, der mir die Möglichkeit gegeben hat, meine Promotion in seinem Arbeitskreis anzufertigen und mich von Beginn an herzlich aufgenommen hat. Ich danke Ihnen für Ihr Vertrauen, Ihre Unterstützung und die stets offenen und konstruktiven Diskussionen zu fachlich relevanten Themen. Auch für die Möglichkeit, zeitweise in den Laboren der HHU arbeiten zu dürfen, bin ich Ihnen sehr dankbar. Ihre fachliche Begleitung war für mich von großem Wert.

Ebenso möchte ich meinem Mentor Jun.-Prof. Dr. Hacker danken für die Übernahme des Koreferats. Vielen Dank für die anregenden Diskussionen zu meinem Promotionsthema im Rahmen der Doktorandenvorträge. Mein Dank gilt auch allen Mitgliedern des Instituts für pharmazeutische Technologie und Biopharmazie, bei denen ich für einige Arbeiten und Messungen zu Gast sein durfte. Trotz des nur kurzen Aufenthaltes habe ich mich sehr wohl gefühlt und von den anregenden Diskussionen auch im Rahmen des Doktorandenseminars und der freundlichen Aufnahme in das Laborumfeld sehr profitiert. Besonders herausheben möchte ich Dr. Björn Fischer für die Diskussionen in der Fokusgruppe Advanced Analytics und seine angebotene Hilfe für den Umzug der HPLCs zur HHU, auch wenn es dazu nicht gekommen ist.

Ein ganz besonderer Dank gilt meinem Chef und externem Betreuer Prof. Dr. Heiko Schiffter-Weinle. Heiko, vielen Dank, dass du mir die Stelle in deinem Arbeitskreis anvertraut hast und mir so die Möglichkeit gegeben hast, an dieser Promotion zu arbeiten. Deine Unterstützung, dein Vertrauen und dein unermüdlicher Einsatz – sowohl in fachlicher Hinsicht als auch persönlich – haben meine Promotionszeit maßgeblich geprägt. Ich bin dankbar für die vielen Gespräche, die Motivation in schwierigen Phasen und das große Engagement, mit dem du mich auf meinem Weg begleitet hast.

Ebenso möchte ich den Studenten der TH Köln danken, die ich während meiner Promotionszeit betreuen durfte. Ihr habt mich nicht nur bei einigen Messungen für diese Arbeit tatkräftig unterstützt, sondern vor allem für eine großartige Atmosphäre im Labor gesorgt. Durch eure Betreuung habe ich selbst viel gelernt. Besonders im letzten Jahr im Chempark-Labor haben wir einen engen Zusammenhalt gepflegt, der die tägliche Arbeit enorm bereichert hat.

Ein herzliches Dankeschön geht an die Mitarbeiter der Fakultät 11, besonders an Anna und Nils, die mir die Einweisung in verschiedene Geräte gegeben haben, vor allem in das NMR und die Feststoffanalytik in Haus 0. Marcus, vielen Dank auch an dich für die Hilfe bei so manchen HPLC-Reparaturen. Ohne eure Hilfe wären viele Experimente so nicht durchführbar gewesen.

Ganz besonders möchte ich mich beim gesamten AK Schiffter bedanken, der mich während meiner Zeit an der TH Köln begleitet hat:

Venosh danke ich für die schöne gemeinsame Zeit im Büro im letzten Jahr. Auch wenn unsere Zusammenarbeit nur kurz war, war sie dafür umso angenehmer. Danke für dein Interesse an meiner Arbeit, dein Durchhaltevermögen in stressigen Zeiten und deine Hilfe bei der Organisation und dem Umzug des Labors. Ich wünsche dir alles Gute für deine Zukunft – du hältst das Labor am Laufen.

Katharina möchte ich danken für die gute Zusammenarbeit und die vielen Gespräche. Ich habe dich zunächst als Kollegin kennen und dann als Freundin schätzen gelernt und habe mich sehr gefreut, dass du der TH für dein Masterstudium und dann für deine Promotion treu geblieben bist. Für deine eigene Promotion wünsche ich dir ganz viel Erfolg.

Ein besonderer Dank geht an Vici, die wohl Dienstälteste im Arbeitskreis. Von Anfang an hast du mir im Labor mit Rat und Tat zur Seite gestanden – sei es bei den ersten Versuchen mit der TFAM-Zelle oder einfach mit einem offenen Ohr zwischen deinen Vorlesungen. Du verbreitest immer gute Laune, auch wenn du es manchmal nicht zugeben willst, und bekommst jeden Gossip mit. Ich wünsche dir alles Gute und viel Erfolg für deine Promotion.

Selma schließlich möchte ich als die gute Seele des Arbeitskreises besonders hervorheben. In unserer gemeinsamen Zeit warst du zunächst Studentin, später Kollegin, in vielerlei Hinsicht auch Mentorin und am allermeisten natürlich eine Freundin. Du hast mich mit deiner ruhigen Art, deinen Ratschlägen und vielen kleinen Aufmerksamkeiten (meist in Form von Schokolade) immer unterstützt. Am Ende unserer gemeinsamen Zeit konnten wir uns oft mit nur einem Blick verständigen. Du verfügst über ein enormes Wissen, auch wenn du es selbst manchmal unterschätzt. Für deinen weiteren Weg wünsche ich dir von Herzen alles Gute, wo auch immer er dich hinführen wird.

All den zuletzt Genannten möchte ich sagen: Bleibt so, wie ihr seid! Ihr seid mir während unserer gemeinsamen Zeit am Campus und im Chempark sehr ans Herz gewachsen. Ich hoffe, wir bleiben auch in Zukunft in Kontakt und sehen uns noch oft wieder – ihr habt meine Promotionszeit zu etwas ganz Besonderem gemacht.

Meinen Eltern und meiner Schwester Michelle danke ich von Herzen für ihre bedingungslose Unterstützung. Ohne euch wären weder mein Studium noch diese Promotion möglich gewesen. Euer Rückhalt, eure Fürsorge und euer Vertrauen haben mir immer die nötige Stärke gegeben, meinen Weg zu gehen.

Zum Schluss gilt mein tiefster Dank Daniel. Danke für deine Zeit, deine Geduld und all die Momente, in denen du mir mit Rat, Zuspruch und liebevoller Unterstützung zur Seite gestanden hast. Du bist immer da, wenn ich dich brauche und ich danke dir dafür von ganzem Herzen.

Eidesstattliche Erklärung

Ich versichere an Eides statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist. Die vorliegende Dissertation wurde bisher bei keiner anderen Fakultät vorgelegt.

Selina Schmidt