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Supersaturating drug delivery systems: the potential of co-amorphous drug formulations

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Graphical abstract
Abstract

Amorphous solid dispersions (ASDs) are probably the most common and important supersaturating drug delivery systems for the formulation of poorly water-soluble compounds. These delivery systems are able to achieve and maintain a sustained drug supersaturation which enables improvement of the bioavailability of poorly water-soluble drugs by increasing the driving force for drug absorption. However, ASDs often require a high weight percentage of carrier (usually a hydrophilic polymer) to ensure molecular mixing of the drug in the carrier and stabilization of the supersaturated state, often leading to high dosage volumes and thereby challenges in the formulation of the final dosage form. As a response to the shortcomings of the ASDs, the so-called co-amorphous formulations, which are amorphous combinations of two or more low molecular weight components, have emerged as an alternative formulation strategy for poorly-soluble drugs. While the current research on co-amorphous formulations is focused on preparation and characterization of these systems, more detailed research on their supersaturation and precipitation behavior and the effect of co-formers on nucleation and crystal growth inhibition is needed. The current status of this research is reviewed in this paper. Furthermore, the potential of novel preparation methods for co-amorphous systems with respect to the current preparation methods are discussed.

Abbreviations

1AFM, atomic force microscopy; ASD, amorphous solid dispersion; AUC, area under curve; C, concentration; Cs, solubility of crystalline drug; DS, degree of supersaturation; DSC, differential scanning calorimetry; EGF, excipient gain factor; FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; FTIR, Fourier-transform infrared spectroscopy; HIF, human intestinal fluid; HPMC, hydroxypropylmethylcellulose; HPMCAS, hydroxypropylmethylcellulose acetate succinate; LLPS, liquid-liquid phase separation; Na–CMC, sodium carboxymethyl cellulose; PG, propylene glycol; PEG, polyethylene glycol; PO, propylene oxide; PVP, polyvinylpyrrolidone; SDDS, supersaturating drug delivery system; SEM, scanning electron microscopy; SI, sink index; SLS, sodium lauryl sulfate; Tg, glass transition temperature; V, volume; USP, Unites states pharmacopea; UV, ultraviolet

Keywords: co-amorphous, supersaturation, dissolution, amorphous solid dispersion

1. Introduction
Efficient drug discovery tools used in the pharmaceutical industry today produce numerous drug development candidates. These drug candidates however, often face serious challenges in their later development into medicines and subsequent market launch (Kalepu and Nekkanti, 2015). Oral formulations of these problematic drugs may fail to deliver the drug to the biological target in the body in a sufficient quantity due to poor biopharmaceutical properties, most often due to poor solubility and slow dissolution of the drug in biological fluids of the gastrointestinal tract (Williams et al., 2013). An estimated 40% of approved drugs and nearly 90% of drug candidates in the development pipeline are poorly water soluble molecules (Loftsson and Brewster, 2010).

Several established and emerging strategies exist to address the poor water solubility problem (Aungst, 2017; Williams et al., 2013). Among these, the so-called enabling, i.e. supersaturating drug delivery systems (SDDS) have attracted increased attention as an effective bioavailability enhancing approach (Fong et al., 2017; Taylor and Chang, 2016). These delivery systems are able to maintain an elevated and sustained level of drug supersaturation in the gastrointestinal fluids which enables improvement of the bioavailability of poorly water-soluble drugs by increasing the driving force for absorption (Sun and Lee, 2013). Amorphous solid dispersions (ASDs) are probably the most common and important SDDS for the formulation of poorly water-soluble drugs (He and Ho, 2015). In an ASD, the drug is ideally molecularly dispersed in an amorphous polymeric carrier to form a glass solution (Grohganz et al., 2014; Vasconcelos et al., 2016). Despite their obvious benefits, the number of commercially available ASDs is relatively low. ASDs often require a high weight percentage of carrier (polymer) to ensure molecular mixing of the drug in the carrier, leading to high dosage volumes and thereby problems in the formulation of the final dosage form (tablet or capsule). In addition, the common carrier polymers are often hygroscopic, which again leads to problems in manufacturing and stability (plasticization by absorbed moisture) (Janssens et al., 2009; Srinarong et al., 2011; Vasconcelos et al., 2016).

As a response to the shortcomings of ASDs, the so-called co-amorphous formulations have emerged as alternative formulations for poorly-soluble drugs (Chieng et al., 2009; Dengale et al., 2016; Grohganz et al.,
These formulations are a combination of two or more low molecular weight components that form a homogeneous amorphous single-phase system. They are classified as a solid-dispersion subtype of glass solutions, with the other forms of glass solutions being polymer- or mesoporous silica-based (Dengale et al., 2016). Combinations of either an active molecule and an excipient (e.g., an amino acid) or two active drug compounds have been shown to enhance the dissolution properties of poorly-soluble drugs and stabilize the amorphous form of both components (Löbmann et al., 2013a; Korhonen et al., 2017). Our review article “Emerging trends in the stabilization of amorphous drugs”, covering the literature before 2013, was the first review presenting achievements of the co-amorphous approach by that time, with future prospects also being discussed (Laitinen et al., 2013). In that review, the research in this field was anticipated to grow and this indeed has happened as reflected by the number of co-amorphous combinations reported today (~50 different drug-excipient combinations in various molar ratios, Korhonen et al. 2017) and review articles about this topic (five according to PubMed search: Chavan et al., 2016; Dengale et al., 2016; Grohganz et al., 2014; Korhonen et al., 2017; Laitinen et al., 2013). In a recent review by Chavan et al. (2016), formulation perspectives and different aspects in the development of co-amorphous formulations as drug products were reviewed. It was stated that while the current research on co-amorphous formulations is focused on preparation and characterization of amorphous systems, more detailed research on the formation mechanism of co-amorphous systems, stabilization and their dissolution benefits is needed. In particular, the precipitation behavior of co-amorphous systems in the solution state and the effect of co-formers on nucleation and crystal growth inhibition, required for a stabilized supersaturated system, need to be investigated. In addition, the need for novel preparation methods, especially those capable of production at an industrial scale, was highlighted.

In this review, our focus is on the evaluation of the supersaturation-ability of co-amorphous formulations. The general concepts and features of SDDS are presented first, after which the dissolution properties of co-amorphous systems and their dosage forms, such as tablets, are reviewed. Finally, we discuss the potential of novel preparation methods for co-amorphous systems with respect to the currently used preparation methods.
2. Supersaturating drug delivery systems

As a consequence of the lack of an ordered crystal lattice, amorphous solids possess enhanced apparent solubility and dissolution properties. This typically leads to drug concentrations in solution that are supersaturated with respect to the thermodynamic equilibrium solubility of the stable crystal form of the respective drug under those particular conditions (Fig. 1). This is referred to the spring effect of dissolution (Brouwers et al., 2009; Williams et al., 2013). This supersaturated state can provide a bioavailability advantage for the drug if it is maintained for a sufficiently long period in the fluids of the gastrointestinal tract. Nucleation and crystal growth of the drug may lead to fast precipitation, unless the rates of these processes are reduced by precipitation inhibitors, such as polymers, or other substances (e.g. cyclodextrins) (Lainé et al., 2016; Palmelund et al., 2016; Williams et al., 2013). The stabilizing effect of precipitation inhibitors is called the parachute-effect (Fig. 1) (Brouwers et al., 2009; Williams et al., 2009). However, if the drug concentration exceeds the amorphous solubility in stabilized supersaturated conditions, i.e. the maximum achievable free drug concentration, and the kinetics of crystallization is slow relative to the dissolution process, a phenomenon called liquid-liquid phase separation (LLPS) may occur (Fig. 1). During LLPS, two phases (a water-rich and an amorphous drug-rich phase) existing in a metastable equilibrium are formed (Ilievbare et al., 2013a; Sun et al., 2016). The drug concentration of the water-rich phase corresponds to the amorphous solubility of the drug while the excess drug precipitates forming a dispersed, colloidal (nano-droplets) amorphous drug-rich phase (Indulkar et al., 2016; Sun et al., 2016). LLPS thus typically occurs under the following conditions: (i) in the presence of precipitation inhibitors, (ii) with weakly basic compounds when the pH of the solution is increased and (iii) upon dissolution of salts (Almeida e Sousa et al., 2016; Indulkar et al., 2015). After LLPS, the solution is still supersaturated with respect to the equilibrium solubility of the stable crystal form and crystallization will eventually occur at some time point (Fig. 1). Occurrence and duration of LLPS is dependent on formulation-related factors, such as drug loading and polymer type (precipitation inhibitor); intrinsic drug properties, such as
amorphous-to-crystalline solubility ratio and crystallization tendency; particle size of the dispersion, and the medium composition and volume (Ilevbare et al., 2013a; Ilevbare et al., 2013b). The formation of a new phase following LLPS results in changes in the appearance and light scattering properties of the solutions (Taylor and Zhang, 2016). This can be utilized in characterization of the LLPS, e.g. detecting the light scattering that arises from the presence of a colloidal phase using ultraviolet (UV)/visible spectroscopy at a non-absorbing wavelength, fluorescence spectroscopy or dynamic light scattering. Specifically, the drug-rich phase can be characterized with cryo-transmission electron microscopy and scanning electron microscopy (SEM) (Ilevbare et al., 2013b; Taylor and Zhang, 2016). The glass transition temperature ($T_g$) of the amorphous material can be measured by differential scanning calorimetry (DSC) to determine if the drug-rich phase is a supercooled liquid or a glass at the temperature of interest (Ilevbare et al., 2013b). For example, the colloidal phase of ritonavir formed upon the dissolution of a ritonavir/PVP (polyvinylpyrrolidone) amorphous solid dispersion was examined by SEM (Ilevbare et al., 2013b). This study revealed that the dispersed phase initially consisted of smooth, spherical liquid-like droplets with an initial size of approx. 500 nm, but coalescence and formation of the appearance of needle-like crystalline particles was visible after approx. 90 min (Fig. 2).

In the following chapters, mechanisms for an efficient parachute effect and implications of precipitation inhibition and LLPS for drug absorption are discussed.

2.1. Mechanisms of precipitation inhibition

The increased chemical potential of a supersaturated system (compared to the corresponding saturated or unsaturated systems) makes it thermodynamically unstable and hence, precipitation will occur at some stage (Palmelund et al., 2016). A precipitation inhibitor can be added to the system to delay or avoid drug precipitation over the required timescale. Depending on the efficiency of the dissolved precipitation inhibitor, drug precipitation from the supersaturated state, and thus the subsequent decline in drug concentration, can be delayed to different degrees (Sun and Lee, 2013; Williams et al., 2013).
Hydroxypropylmethylcellulose acetate succinate (HPMCAS) has been identified as the most effective polymer in achieving and maintaining drug supersaturation among several available water-soluble polymers typically used as carriers in ASDs (Friesen et al., 2008; Sun and Lee, 2013). Table 1 presents the most important mechanisms of precipitation inhibition. However, it should be noted that the efficiency and mechanisms of precipitation inhibition not only depend on the inhibitor type, but also on its concentration in the media, the drug with which it is used and other excipients in the formulation (Brouwers et al, 2009; Mah et al., 2016; Palmelund et al., 2016). For example, in a study with phenytoin and different derivatives of this drug, hydroxypropylmethylcellulose (HPMC) was found to maintain drug supersaturation solely by drug crystallization inhibition, whereas PVP maintained the drug supersaturation by both a drug solubilization effect and inhibition of crystallization, depending on the drug compound (Otsuka, et al., 2015). Furthermore, it has been suggested that in order to be an effective crystallization inhibitor in aqueous solutions, a polymer needs to be suitably hydrophobic. If it is too hydrophilic, it will preferably interact with water and if it is too hydrophobic, it will preferably interact with itself, rather than with the nucleating drug phase (Ilevbare et al., 2013a; Schram et al., 2015). This is supported e.g. by the observation that the very hydrophilic polyacrylic acid was unable to inhibit nucleation of supersaturated celecoxib in contrast to the less hydrophilic polymers HPMC and HPMCAS (Xie and Taylor, 2016). Also the nature of the media in which precipitation occurs affects the process, and thus the relative abilities of different excipients to prevent crystallization may vary depending on the dissolution media (Williams et al., 2013).

2.2. Supersaturation ability of formulations: investigation and implications for absorption

Dissolution studies for supersaturating systems should be conducted in non-sink conditions (Sun and Lee, 2015). However, the extent of these non-sink conditions should be considered carefully when designing a dissolution test for a supersaturating dosage form. For quantitative evaluation of the deviation of the dissolution conditions from sink-conditions, a sink index (SI) has been developed (Eq.1):

\[ SI = \frac{C_s}{Dose/V} \]
where $C_s$ is the solubility of crystalline drug, $V$ the volume of dissolution medium, and Dose is the total amount of drug in the sample (Sun et al., 2012). The larger the SI, the closer to the sink condition, i.e. SI > 3 corresponds to the USP definition of sink conditions and when SI is larger than 10, a perfect sink condition is achieved (Han and Lee, 2017). The SI of a particular dissolution test has a strong influence on the resulting kinetic solubility profile of a drug from a formulation. For example, polymers are more effective precipitation inhibitors at low levels of supersaturation than at high levels of supersaturation (Han and Lee, 2017; Ilevbare et al., 2012).

In addition, deviation from sink conditions can also be described by the degree of supersaturation (DS), which can be defined as the ratio between the concentration ($C$) of the supersaturated system and the thermodynamic equilibrium solubility of the stable crystalline form of the compound (Eq.2):

$$DS = \frac{C}{C_s}$$  

(2)

The mechanism and kinetics of precipitation inhibition can be studied by precipitation studies, where supersaturation is not a consequence of dissolution of a formulation, such as an ASD, but can be induced e.g. by a pH- or solvent-shift method (Bevernage et al., 2013). In the solvent-shift method, a compound is dissolved in an organic, water-miscible solvent and then a certain volume of the solution is added into an aqueous medium to achieve a desired DS by adjusting the drug concentration in the organic solution and/or the transferred amount. Supersaturation and/or precipitation can be then monitored by analyzing the drug concentrations at specific time points and forming a concentration vs. time or DS vs. time profile (Bevernage et al., 2011). Alternatively, a shift in pH that results in reduced ionization will rapidly decrease the drug solubility (as expressed in the Henderson–Hasselbalch equation) and induce a supersaturated state for ionizable drugs. In the precipitation experiments, the efficiency of precipitation inhibitors in stabilizing supersaturation at a specific DS-level can be assessed by calculating the excipient gain factor (EGF) for each individual inhibitor (Eq. 3):

$$EGF = \frac{AUC_e}{AUC}$$  

(3)
where $AUC_e$ is the area under curve up to a selected time point of the DS-time profile in the presence of an excipient and $AUC$ is the $AUC$ of the DS-time profile in the absence of excipients (Bevernage et al., 2011).

Careful consideration of the hydrodynamics of the test (e.g. mixing or shaking), medium selection (buffer, simulated gastrointestinal fluids etc.) and temperature should be conducted when selecting conditions for dissolution/precipitation testing, as all these may affect the nucleation and growth rates (Bevernage et al., 2013). Palmelund et al. (2016) used fasted-state simulated intestinal fluid (FaSSIF) to investigate supersaturation and precipitation of six model drug compounds in four different concentrations in a small-scale setup ($\mu$DISS Profiler™). From the time-concentration profiles it was observed that the induction time of nucleation was dependent on the initial supersaturated concentration (DS), i.e. lower concentrations systematically yielded longer induction times. The model compounds also showed different tendencies to supersaturate, i.e. albendazole, fenofibrate and felodipine were found to be able to keep a supersaturation with a DS around 2 for 60 min, while aprepitant and tadalafil could stay supersaturated for the same time with a DS around 5 and danazol with a DS of 7.5. The study demonstrated that it is necessary to study supersaturation using several DS values for each compound in order to get an understanding of their tendency to supersaturate and avoid misleading data resulting from the use of a specific experimental setup. When the mechanisms of precipitation inhibition are of interest, they can be studied experimentally by different approaches. For example, drug-inhibitor interactions are likely to stabilize the supersaturated state, if the drug and the inhibitor have been found to be miscible and interactions have been observed in the solid-state by Fourier-transform infrared spectroscopy (FTIR) or Raman spectroscopy (Baghel et al., 2016; Chauhan et al., 2013). Increasing ability to stabilize supersaturation by the increasing molecular weight of a polymer can indicate that increased solution viscosity may be the stabilizing mechanism, which can be evaluated by viscosity measurements (Miller et al., 2008). Instead, testing different grades (molecular weight and/or hydrophobic substitution) of a polymer, e.g. HPMC and HPMCAS, on the stabilization of itraconazole revealed that stabilization of the drug in supersaturated state was related to the molecular weight of the polymer and the degree of hydrophobic substitution of HPMCAS, indicating that stabilization was achieved through a combination of steric hindrance and hydrophobic interaction.
SEM can reveal the possible adsorption of the inhibitor on the surfaces of the nuclei. Ghosh et al. (2011) observed that HPMC interacted more strongly with the model drug compared to PVP K-30 and adsorbed more efficiently onto drug crystals to provide steric stabilization of the supersaturated state. Atomic force microscopy (AFM) can also be used for studying adsorption of inhibitors onto drug surfaces (Schram et al., 2015). In the case that stabilizing, colloidal drug-polymer nanoassemblies are formed, they can be separated from a precipitate using centrifugation and from dissolved species using dialysis and further characterized by dynamic light scattering and cryogenic transmission electronic microscope imaging (size and structure) (Friesen et al., 2008).

Correlating the in vitro dissolution/precipitation study results with the in vivo absorption and bioavailability advantage remains challenging for supersaturating formulations. The in vitro dissolution set-up may not be predictive of precipitation in in vivo conditions or may not be able to take into account the increased absorption, generated by an increased driving force due to drug supersaturation. Moreover, the stabilization mechanisms of drug supersaturation may also affect drug permeability. Formation of complexes or micelle systems between a drug and a solubilizing precipitation inhibitor reduces the concentration of free drug in the solution and thus decreases the apparent permeability of the drug. For example, PVP was found to result in decreased Caco-2 cell permeability of diphenylhydantoin (Otsuka et al., 2015). In addition, fast dissolution of supersaturating formulations does not always translate into an improved membrane permeability or in vivo performance (Augustijns and Brewster, 2012; Otsuka et al., 2015). Sun and Lee (2013) determined experimental concentration–time curves with varying rates of supersaturation generation and recrystallization for indomethacin, naproxen and piroxicam in the absence of precipitation inhibitors. The results were compared with those predicted from a comprehensive mechanistic model based on classical nucleation theory taking into account both the particle growth and ripening processes. Both the experimental and predicted results showed that when the rate of supersaturation generation (i.e. the drug dissolution rate) increased, the maximum achievable supersaturation (i.e. the kinetic solubility) of the amorphous solids increased, the time to reach maximum supersaturation decreased, and the rate of concentration decline after maximum supersaturation
increased. Thus, the AUC of the kinetic solubility concentration–time profile also decreased, which would not be beneficial in vivo. Furthermore, it was possible to model and experimentally verify an optimal supersaturation rate for indomethacin, naproxen and piroxicam, which would maximize the AUC and thus the dissolution advantage. It was shown that an optimal AUC, resulting in an optimal in vivo performance, of the in vitro concentration–time profile exists at an intermediate supersaturation rate (or a modest in vitro dissolution rate).

When supersaturation occurs in vivo without solubilization, the drug can exhibit higher flux-values through the intestinal membrane than those obtained from solubilized systems at same concentrations, as the flux depends on thermodynamic activity of the solute (Indulkar et al., 2016). The flux across a membrane can increase until the amorphous solubility is reached; above this, any further increases in concentration do not lead to an increase in thermodynamic activity or flux. However, it is known that when supersaturation occurs rapidly and precipitation inhibitors are present, the drug may undergo LLPS at concentrations that exceed the amorphous solubility (Jackson et al., 2016). LLPS can be advantageous in vivo, as the drug-rich phase formed when exceeding the amorphous solubility can serve as a reservoir replacing the drug permeating through a biological membrane at the maximum flux in the solution phase (Raina et al., 2014). This has been observed e.g. with clotrimazole in phosphate buffer (pH 8) in a flow-through diffusion system with a cellulose membrane (Indulkar et al., 2016). In this setup, concentrations above the amorphous solubility led to the formation of clotrimazole-rich nanodroplets with a size of approximately 200 nm. This phase provided a reservoir that rapidly replenished the free drug removed by permeation across the membrane and thus, solutions that contained clotrimazole nanodroplets showed a maximized and sustained (i.e. for as long as LLPS existed) membrane transport rate.

Prediction of the in vivo performance of supersaturating systems by in vitro testing remains challenging (Gao and Shi, 2012). The gastrointestinal environment affects drug precipitation in vivo and thus a biorelevant in vitro evaluation of supersaturation requires careful selection of the test medium (Bevernage et al., 2011; Tsume et al., 2017). Bevernage et al. (2011) studied supersaturation of a range of poorly water-
soluble drugs (etravirine, ritonavir, loviride, danazol and fenofibrate) in human intestinal fluids (HIF) representing both the fasted and fed state using a solvent-shift method. HPMCAS, HPMC-E5, HPMC-E50, HPMC-E4M, HPMC-P and PVP at a DS value of 20 were used as precipitation inhibitors. It was found that excipient-mediated precipitation inhibition was less pronounced in HIF compared to simple aqueous buffer or FaSSIF/FeSSIF (fed-state simulated intestinal fluid). In a study with HPMCAS-based posaconazole ASD tablets, Chen et al. (2016a) investigated the impact of sodium lauryl sulfate (SLS), which is often used as a solubilizer and/or wetting agent in various dosage forms, on their dissolution behavior and in vivo bioavailability. They observed that HPMCAS incorporated into ASD was able to significantly delay the crystallization of posaconazole in the drug-rich amorphous phase, formed as a consequence of LLPS, from less than 10 min to more than 4 h. However, when SLS was added into the formulation, the dissolution rate was found to be enhanced, but SLS negated the crystallization inhibition effect of HPMCAS in the posaconazole-rich amorphous precipitates and thus caused fast drug crystallization within 30 min. These in vitro results correlated well with the results of an in vivo dog pharmacokinetic study, where the bioavailability of HPMCAS-based ASD formulations with or without SLS was compared. Strikingly, the formulation without SLS showed a significantly higher bioavailability, i.e. $C_{\text{max}}$ and AUC of approximately 2.5 and 3.4 fold higher than that of the formulation with SLS. These results demonstrated that the in vivo performance of the ASD-based formulations was better correlated with the fate of the entire dose of the drug in the tablet, i.e. both the dissolved drug and the precipitated drug, than only the drug dissolution rate. It also showed that the interplay between the components in the formulation could critically affect each process occurring in the solution and the ultimate in vivo bioavailability.

3. Supersaturation in co-amorphous formulations

In co-amorphous systems, different types of co-formers have been combined with an active drug molecule: another drug (combination therapy), amino acids, citric acid, saccharin etc. as reviewed by Dengale et al., 2016; Grohganz et al., 2014; Korhonen et al., 2017; Laitinen et al., 2013. Co-amorphous systems have been
found to improve dissolution behavior of drugs when compared to their crystalline counterparts (physical mixtures) and most importantly, individual amorphous forms (Allesø et al., 2009; Lenz et al., 2015; Löbmann et al., 2011a). However, most often dissolution studies for co-amorphous systems have been conducted with intrinsic or other sink-condition methods (Korhonen et al., 2017), thus information on the supersaturation ability of these systems in aqueous solutions often remains unexplored. Nevertheless, there are a few studies which are helpful in assessing the supersaturation ability of co-amorphous mixtures (Table 2).

3.1. Co-amorphous salts

As can be seen from Table 2, supersaturation of drugs from co-amorphous formulations has been observed both with drug-drug and drug-inactive co-former combinations. From a dissolution and supersaturation point of view, amorphous salts are particularly interesting, since they have both, a higher solubility of the ionized form in comparison to the unionized form of the drug and a higher apparent solubility due to being in an amorphous form, which might provide an effective enhancement of dissolution in biological fluids (Guerrieri et al., 2010). Paluch et al. (2010) prepared amorphous ciprofloxacin-succinic acid salts at a 1:1 molar ratio by spray drying from water and at a 2:1 molar ratio by ball milling. In dynamic solubility testing in water at 25°C and 37°C, the amorphous 2:1 salt had a superior solubility behavior when compared to the 1:1 amorphous salt and crystalline salts at different stoichiometric ratios. The co-amorphous salt produced a long-lasting drug supersaturation, with the highest ciprofloxacin concentration of 58.8±1.18 mg/ml after 1 h of the experiment at 37°C (the corresponding value for crystalline drug was smaller than 0.1 mg/ml).

It has also been shown that indomethacin and arginine form a co-amorphous salt at a molar ratio of 1:1, irrespective of the preparation method used (Jensen et al., 2016; Löbmann et al., 2013b). When the dissolution properties of tablets consisting of mannitol, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate and indomethacin-arginine in either physically mixed or co-amorphous (spray-dried) form were tested in non-sink conditions at pH 4.5, the tablets containing the co-amorphous indomethacin-arginine showed a fourfold drug supersaturation when compared to the solubility of crystalline
indomethacin (Lenz et al., 2015). Increasing the compaction forces used in tableting resulted in a slight decrease in the maximum supersaturation concentration due to the slower erosion of tablets with a higher tensile strength, but the AUC\textsubscript{24h} was not affected (threefold higher than for the tablet with crystalline indomethacin). The dissolution properties of the tablets containing the co-amorphous mixture were found to remain unaffected after storage at 40 °C over silica gel for 12 months. Interestingly, when the tablets containing the physical mixture of drug and amino acid were tested, a color change in the tablet from white to yellow was observed already after a few minutes into the test, indicating \textit{in situ} amorphization of indomethacin (Priemel et al., 2013). As a consequence, an approximately threefold supersaturation was observed. However, recrystallization of the dissolved drug also occurred much faster than for the tablets containing the co-amorphous mixture. Furthermore, the plateau concentrations after 24 h of dissolution differed and this was explained by the formation of different polymorphic forms of indomethacin in the precipitates (i.e. Y form from tablets containing the physical mixture, and an unknown metastable form from tablets containing the co-amorphous mixture).

### 3.2. Supersaturation of co-amorphous systems in biorelevant media

The supersaturation of co-amorphous indomethacin-arginine was further studied in detail by Ojarinta et al. (2017) (Table 2). Dissolution and precipitation studies were conducted in fasted state simulated intestinal fluid (FaSSIF, pH 6.5) and fed state simulated intestinal fluid (FeSSIF, pH 5.0) and their corresponding blank buffers (i.e. without phospholipid and taurocholate). In addition to indomethacin-arginine, the co-amorphous mixtures of the drug with phenylalanine and tryptophan as co-formers were also investigated. All mixtures were prepared by cryomilling. To the best of our knowledge, this was the first and currently the only study systematically exploring the efficiency of co-formers as precipitation inhibitors in biorelevant conditions. Dissolution studies for crystalline indomethacin and physical mixtures with the amino acids revealed that only arginine was able to significantly improve indomethacin dissolution from physical
mixtures when compared to crystalline drug in FaSSIF blank, FeSSIF and FaSSIF. For the physical mixture of indomethacin and arginine, a clear supersaturation was seen before reaching a concentration plateau. The improved dissolution behavior was explained by salt formation, an interaction between the guanidinium group of arginine and the aromatic groups of indomethacin, in situ amorphization and the subsequent formation of an amorphous indomethacin-arginine salt (ElShaer et al., 2011; Lenz et al., 2015). In the case of amorphous indomethacin and co-amorphous mixtures (Fig. 3), a clear indomethacin supersaturation was observed from indomethacin-arginine in FaSSIF blank and, to lesser extent, FaSSIF, FeSSIF blank and FeSSIF. The dissolution profiles of the other co-amorphous mixtures were similar to that of amorphous indomethacin. This was explained by the lack of strong interactions between the mixture components. However, EGF values (i.e. comparison of AUC0-72h between co-amorphous indomethacin-amino acid mixtures and amorphous indomethacin) were calculated based the results of the dissolution studies and they indicated a statistically significant, albeit modest (EGFs < 2), inhibitory effect on drug precipitation by the amino acids, except for phenylalanine in FeSSIF blank and FaSSIF and for tryptophan in FaSSIF. Arginine showed the highest EGF-value in FaSSIF (>10), indicating a significant stabilization of supersaturation. In the precipitation studies, performed by the solvent-shift method with an DS of 4 (Fig. 4), arginine better maintained indomethacin supersaturation in FaSSIF blank and FaSSIF, with EGF-values (i.e. comparison of AUC0-360min) between amino acid-indomethacin combinations and pure indomethacin of >2 and >3, respectively. The other amino acids and arginine in other conditions did not show any inhibitory effect on indomethacin precipitation (EGF-values ~1). Thus, the extent of supersaturation seemed to depend on the media and the interactions between the drug and amino acids. With arginine, the higher pH of FaSSIF blank and FaSSIF, which led to the ionization of indomethacin, promoted electrostatic interactions between the drug and arginine and thus led to salt formation. At a pH of 5.0, indomethacin was less ionized and thus the supersaturation may have been maintained mainly by interactions between the guanidinium group of arginine and the aromatic moiety of indomethacin (Hirano et al., 2010; Hirano et al., 2013; Shah et al., 2012). Furthermore, biorelevant media exerted a significant effect on indomethacin solubility, i.e. in the lower pH of FeSSIF, where indomethacin was more unionized and thus more solubilized by the bile salt
micelles, the stabilizing effect of arginine was outweighed by solubilization due to the medium components.

Similar effects of the dissolution medium were observed in the study by Heikkinen et al. (2015) (Table 2). Supersaturation of glibenclamide and simvastatin and their co-amorphous formulations with different amino acids was observed in phosphate buffer and to a lesser extent in biorelevant media. This suggests that the dissolution advantage observed in aqueous buffers may be an overestimation of the dissolution advantage in vivo. For the neutral drug simvastatin, a significant drug supersaturation was observed from simvastatin-lysine 1:1 co-amorphous formulations in FaSSIF, but in FeSSIF this was overruled by the large solubilizing effect of the high concentration of bile salts. In the case of glibenclamide, drug supersaturation in biorelevant media was less evident compared to that occurring in buffer. Additionally, clear differences in the supersaturation behavior of the co-amorphous formulations and amorphous drug alone were not observed.

3.3. Supersaturation behavior of poorly water-soluble drug mixtures

Supersaturation has been observed for poorly water soluble drug-drug combinations, such as atorvastatin calcium–carvedilol, atorvastatin calcium–glibenclamide and simvastatin–glipizide, but the observed improvement has been quite modest (Table 2). Co-formers may not always have a positive impact on the drug supersaturation and possibly drug absorption through membranes. Trasi and Taylor (2015a) studied the supersaturation in phosphate buffer and membrane permeation (though a semipermeable regenerated cellulose dialysis membrane) for single component aqueous solutions of ritonavir, lopinavir and paclitaxel. They found that the maximum flux through the membrane was limited by the amorphous solubility of the drug and that exceeding this concentration lead to the formation of a drug-rich phase (LLPS) with no further increase in the flux. Interestingly, the maximum flux for these compounds was significantly decreased when a second component was dissolved into the medium if the components present were miscible in the amorphous drug-rich phase, i.e. with ritonavir–paclitaxel and ritonavir–lopinavir combinations. Thus, the amorphous solubility of that compound was reduced by the presence of the
second component. More specifically, addition of a small amount of lopinavir to a solution containing a ritonavir-rich phase lead to favorable mixing of lopinavir with the ritonavir-rich phase rather than with water, leading to a decrease in the bulk solution concentration. The results may have implications for co-formulation of two or more amorphous drugs, i.e. co-amorphous formulations, which may result in a reduction of the amorphous solubility of each component and hence a lower achievable supersaturation with a consequent reduction in the rate of membrane transport. Dissolution properties of the ritonavir-lopinavir combination were further studied in pH 6.8 buffer when the combination was formulated as an amorphous solid dispersion with PVP or HPMCAS (Trasi and Taylor, 2015b). It was found, that supersaturation was achieved with all combination ASDs, but the maximum achievable concentration of each drug was much lower compared to those achieved with the individual dispersions. Although the reduced maximum achievable supersaturation can be considered a drawback, it may, on the other hand, be advantageous in reducing the driving force for crystallization and thus both of these effects should be considered when evaluating implications for bioavailability. Similar results were obtained for ASDs containing ritonavir and atazanavir and ritonavir, atazanavir and lopinavir at different molar ratios (Alhalaweh et al., 2016). A formulation containing a 1:1 molar ratio of ritonavir and atazanavir achieved only half of the supersaturation attained by dissolution of the single drug systems and, for the ternary dispersion, the maximum concentration of each drug was only one third of that achieved for the single drug ASD formulations. This was explained by a decrease in the drug concentration at which the LLPS occurs in the presence of other miscible drugs, which reduced the maximum achievable supersaturation of each drug. This, in turn, decreased the observed flux across Caco-2 cells for the drug combinations compared to drug alone. From these studies it can be concluded that a decrease in maximum achievable supersaturation is likely to be of importance for the dissolution and absorption of multicomponent amorphous dosage forms, particularly those containing combinations of poorly water soluble drugs, which may undergo supersaturation in vivo (Alhalaweh et al., 2016; Trasi and Taylor, 2015b).

From the above discussion it can be concluded that co-amorphous salt formation has been proven effective in stabilizing supersaturation. Amorphous salt formation may thus have the best potential for precipitation inhibition of the co-amorphous formulations, as, for example, arginine showed a significant inhibitory effect for indomethacin precipitation in FaSSIF (Ojarinta et al., 2017). However in future, further in vitro evaluation under biorelevant conditions would be necessary to extrapolate in vitro supersaturation assessment to the in vivo situation. In addition, the mechanism of the possible precipitation inhibition of the co-formers requires further investigations. Theoretically the most plausible precipitation inhibition mechanism for co-amorphous excipients would be interaction with the drug in solution. This is supported by the studies where supersaturation has been most efficiently stabilized when strong intermolecular interactions have been detected between the components, i.e. amorphous salt formation (Ojarinta et al., 2017; Paluch et al., 2010) or hydrogen bonding (Wang et al., 2017). It is also possible that adsorption on the “surfaces” of nuclei and, in some cases solubilization, may inhibit drug recrystallization. As the active drug compounds in co-amorphous formulations are often poorly soluble and hydrophobic, it may well be that, similar to polymers, the co-former also needs to be sufficiently hydrophobic to be able to inhibit recrystallization by surface adsorption and interaction with the drug molecules (other than salt formation) instead of interacting with water (if too hydrophilic) or with itself (if too hydrophobic) (Ilevbare et al., 2013a; Schram et al., 2015). Increased medium viscosity as a mechanism of precipitation inhibition in the case of small molecule excipients can be considered unlikely.

4.1 Novel approaches for preparation of co-amorphous formulations

There are only a few studies investigating the use of co-amorphous formulations in dosage forms. Generally co-amorphous systems are intended for oral administration as tablets or capsules (Chavan et al., 2016; Lenz et al., 2015; Renuka et al., 2017). Current preparation methods, i.e. quench-cooling, milling and solvent evaporation methods, are mostly not scalable to manufacturing scales (except spray-drying). In addition, they may produce powders that do not have ideal properties (such as poor powder flow or poor
compressibility) for conversion into final dosage forms (Chavan et al., 2016; Dengale et al., 2016). Thus, it would be interesting to investigate alternative preparation methods to improve the feasibility of co-amorphous mixtures in pharmaceutical products, and to consider dosage forms other than tablets and capsules.

*In situ* amorphization can be achieved via a dissolution or vaporisation process and via lipolysis (Priemel et al., 2016). Often these processes occur too slowly from a pharmaceutical viewpoint, but relatively fast *in situ* amorphization has been observed for indomethacin, ibuprofen and naproxen with Eudragit® E (Doreth et al., 2016; Priemel et al., 2013), and for indomethacin-arginine tablets (Chapter 3.1) during dissolution and during storage at high humidity conditions (room temperature/75% relative humidity) (Petry et al., 2017). This offers interesting possibilities to convert a formulation into the amorphous form just prior to application, thus achieving a dissolution advantage but avoiding stability problems of an amorphous formulation during storage. With the aid of microwave radiation, *in situ* amorphization has been obtained even faster. Doreth et al. (2017) used a conventional household microwave oven for (partial) amorphization of humidity-treated indomethacin-PVP K12 tablets. Humidity conditioning before microwaving provided absorbed water in the tablets which was necessary to absorb the energy from the microwaves. Higher relative humidity (54%), higher microwave power (1000W) and longer microwaving time (90s) were found to lead to the highest degree of amorphization of indomethacin (approx. 80%) in the tablets. These tablets, despite of their residual crystallinity, showed a similar intrinsic dissolution behaviour as the fully amorphous indomethacin:PVP glass solution. The concept of *in situ* amorphization in the microwave oven may in future allow to prepare amorphous formulations and circumvent the physical stability issues during long-term storage, and would be worth investigating with co-amorphous formulations, especially considering the *in situ* amorphization potential of indomethacin-arginine. Furthermore, other high-energy irradiation could be used for generating amorphous forms, although these may not be available for consumers or suitable for large scale production. For example, laser irradiation has been found able to transform indomethacin powder from the crystalline to an amorphous form, which was more stable upon storage than a melt-quenched reference material (Titapiwatanakun et al., 2016).
Spray-drying has been used as a preparation method for co-amorphous formulations intended for tablets or capsules and it should also be noted that this is a convenient method for the preparation of powders for inhalation (Chen et al., 2016b). Amino acids have been used as excipients in different kinds of powders for inhalation, i.e. with macromolecular and small-molecule drugs, and leucine is the most commonly employed. Leucine has surface active properties and when co-spray dried with drugs, it has been found to result in a dry particle with a crystalline leucine shell (Boraey et al., 2013) e.g. in leucine-budesonide formulations. When combined with macromolecular drugs or other excipients, leucine has also been found to be amorphous in these powders (Chen et al., 2016b). However, to the best of our knowledge, there are no reports of co-amorphous formulations intended for inhalation. Perhaps in the future spray-dried co-amorphous formulations could also be employed in inhalers.

Fast-dissolving oral films, claimed to contain a model drug in a co-amorphous form with a carboxylic acid, have also been prepared (Maher et al., 2016). A co-amorphous mixture of olanzapine and ascorbic acid was first prepared by rapid solvent evaporation and subsequent characterization showed co-amorphization of the components through hydrogen bonding at 1:1 and 1:2 molar ratios, of which the 1:2 mixture better improved in drug dissolution. A fast dissolving film, containing olanzapine:ascorbic acid at a 1:2 ratio and HPMC and sodium carboxy methyl cellulose (Na–CMC) as film-forming polymers, glycerin, propylene glycol (PG), or polyethylene glycol (PEG) 400 were used as plasticizers, citric acid as a saliva stimulant, Na-saccharine as a sweetening agent, and menthol as a flavoring agent, was prepared by solvent casting. The optimized film-formulation showed complete drug dissolution in 10 minutes which was a significant improvement in the dissolution rate compared to plain olanzapine powder. Improved in vivo bioavailability was also observed for the film when compared to the commercially available tablet formulations (Olazine oral tablets from EIPICO and fast dissolving freeze-dried tablet Zyprexa® Velotab from Lilly). However, it remains somewhat unclear if a co-amorphous drug-ascorbic acid mixture was actually present in the films, since interactions between the components in the films were not assessed and dissolution was only compared with plain drug powder. Since the films were prepared by dissolving the film-forming polymer and subsequently the co-amorphous powder in water, it would have been essential to compare this with a
situation in which crystalline drug and ascorbic acid would have been added to the polymer solution, to see if there was any difference to the co-amorphous powder and if a co-amorphous system between the drug and the acid was actually formed (in one or both cases). In contrast, for indomethacin-arginine, formation of a co-amorphous system was confirmed by FTIR when inkjet printing an ink solution (dimethyl sulfoxide, propylene glycol and water) containing indomethacin and arginine in 1:1 molar ratio onto an impermeable transparency film using a piezoelectric inkjet printer (Wickström et al., 2015). These formulations, containing no polymeric excipients, showed an enhanced dissolution rate over a crystalline reference, and could offer an interesting means for preparation of personalized drug delivery systems (oral and/or orodispersible), provided that the challenges associated to the printer, printhead technology, the ink and substrate properties can be overcome (Planchette et al., 2016; Wickström et al., 2015).

5. Conclusions

In this review, we have evaluated the supersaturation ability of co-amorphous systems. In amorphous solid dispersions, polymers are well known precipitation inhibitors and they are able to stabilize the supersaturated state of the drug by different mechanisms, which may improve oral bioavailability, if the supersaturated state is maintained over a sufficient time period to allow drug absorption through the intestinal wall. In co-amorphous systems the excipients are small molecules, e.g. drug molecules or amino acids. Thus, their ability to induce and stabilize drug supersaturation is inherently different to polymers. Drug supersaturation has been observed to occur from both drug-drug and drug-excipient co-amorphous systems in different dissolution media, but detailed investigation of the supersaturation kinetics and mechanism of stabilization are needed in future. Furthermore, the components of a co-amorphous mixture may have unpredictable and unwanted effects, such as a decrease in maximum achievable supersaturation which may have significant impacts on dissolution and absorption in vivo and thus potential for enhancing drug bioavailability, particularly from co-amorphous drug-drug combinations.
Co-amorphous formulations are most commonly intended to be formulated as tablets for oral use. However, depending on the drug, they are also suitable for other administration routes, such as the buccal or pulmonary routes. In addition, printing of co-amorphous formulations on edible substrates may offer an interesting approach for preparation of personalized drug delivery systems in the future.

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http://dx.doi.org/10.1016/j.addr.2016.01.012


### Table 1. Mechanisms for precipitation inhibition in supersaturating formulations

<table>
<thead>
<tr>
<th>Precipitation inhibition mechanism</th>
<th>Excipients</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubilization</td>
<td>Surfactants, emulsifiers, polymers</td>
<td>Reduction of thermodynamic drivers of precipitation by increase in apparent solubility. Some polymers, such as PVP, are able to solubilize drugs. Micelle formers (surfactants, Soluplus®) can solubilize drugs inside the micelles. For example, formation of a Soluplus*-sodium dodecyl sulfate complex significantly improved the solubilization and stabilization of a supersaturated solution of the poorly water-soluble drug cyclosporine A.</td>
<td>Li et al., 2012; Otsuka et al., 2015; Williams et al., 2013; Xia et al., 2016</td>
</tr>
<tr>
<td>Interaction with drug in solution</td>
<td>Polymers, surfactants</td>
<td>Drug-polymer interactions lead to inhibition of nucleation. For example, supersaturation of dipyridamole and cinnarizine was stabilized more efficiently by stronger drug-polymer interactions with polyacrylic acid than with polyvinylpyrrolidone. In addition, Pluronics with a propylene oxide (PO) block length &gt;3000 Da, may interact with the drug through the PO blocks and thus inhibit precipitation.</td>
<td>Baghel et al., 2016; Chauhan et al., 2013; Li et al., 2012. Palmelund, et al., 2016</td>
</tr>
<tr>
<td>Adsorption to the interface</td>
<td>Polymers, surfactants</td>
<td>Adsorption to the interface of nuclei slows down crystal growth.</td>
<td>Palmelund, et al., 2016</td>
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<tr>
<td>Event</td>
<td>Polymers</td>
<td>References</td>
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<tr>
<td>Polymer hydrophobicity has been found to impact polymer adsorption</td>
<td>Polymer hydrophobicity has been found to impact polymer adsorption</td>
<td>2016; Schram et al., 2015; Xie and Taylor, 2016</td>
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<td>and adsorbed polymer conformation, which in turn impacts its surface</td>
<td>and adsorbed polymer conformation, which in turn impacts its surface</td>
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<td>coverage. This has been shown to directly correlate to the polymer’s</td>
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<td>effectiveness as a growth rate inhibitor. For example, HPMCAS has</td>
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<td>been found to be an effective inhibitor of celecoxib crystal growth at</td>
<td>been found to be an effective inhibitor of celecoxib crystal growth at</td>
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<td>both high and low levels of supersaturation.</td>
<td>both high and low levels of supersaturation.</td>
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<td>Increased medium viscosity  Polymers</td>
<td>Increased medium viscosity  Polymers</td>
<td>Miller et al., 2008</td>
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<td>Higher molecular weight polymers (e.g. HPMC) were found to be more</td>
<td>Higher molecular weight polymers (e.g. HPMC) were found to be more</td>
<td>Miller et al., 2008</td>
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<td>efficient stabilizers of supersaturation than lower molecular weight</td>
<td>efficient stabilizers of supersaturation than lower molecular weight</td>
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<td>equivalents. An increase in the local viscosity surrounding the</td>
<td>equivalents. An increase in the local viscosity surrounding the dissolving</td>
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<td>dissolving drug (itraconazole) by the higher molecular weight polymer</td>
<td>dissolving drug (itraconazole) by the higher molecular weight polymer</td>
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<td>delayed diffusion of the solubilized drug into bulk solution. This</td>
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<td>allowed for the drug to remain in intimate association with the</td>
<td>allowed for the drug to remain in intimate association with the</td>
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<td>stabilizing polymer, with intermolecular interactions between the</td>
<td>stabilizing polymer, with intermolecular interactions between the drug and</td>
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<td>drug and the polymer providing stabilization of the drug in the</td>
<td>drug and the polymer providing stabilization of the drug in the</td>
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<td>thermodynamically unfavorable aqueous environment.</td>
<td>thermodynamically unfavorable aqueous environment.</td>
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<tr>
<td>Formation of structured Polymers</td>
<td>The hydrophilic regions of the amphiphilic polymer HPMCAS allow drug-</td>
<td>Friesen et al., 2008</td>
<td></td>
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<tr>
<td>nanoassemblies (colloids)</td>
<td>polymer structures to remain as stable colloids in aqueous solution.</td>
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</table>
Table 2. Co-amorphous systems for which supersaturation has been investigated in non-sink conditions

<table>
<thead>
<tr>
<th>Co-amorphous combination, molar ratio</th>
<th>Supersaturation</th>
<th>Mechanism of precipitation inhibition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin calcium–carvedilol 1:1</td>
<td>Fast supersaturation over crystalline solubility (&gt; twofold) for both components in water. Stable for 4h (carvedilol) and 5 days (atorvastatin). More stable supersaturation confirmed by comparison with results for pure amorphous drugs from other studies.</td>
<td>NA</td>
<td>Shayanfar and Jouyban, 2013</td>
</tr>
<tr>
<td>Atorvastatin calcium–glibenclamide 1:1</td>
<td>Approx. twofold supersaturation over crystalline solubility achieved in 2 h for both drugs. More stable supersaturation confirmed by comparison with results for pure amorphous drugs from other studies.</td>
<td>NA</td>
<td>Shayanfar and Jouyban, 2013</td>
</tr>
<tr>
<td>Ciprofloxacin–succinic acid 1:1</td>
<td>An approximately 1000-fold supersaturation compared to the crystalline drug was reached in water after 1 h at 37°C. Liquid–liquid phase separation possibly occurred.</td>
<td>Amorphous salt formation</td>
<td>Paluch et al., 2013</td>
</tr>
<tr>
<td>Repaglinide–saccharin 1:1</td>
<td>Repaglinide supersaturation was achieved in water in 2 h with further increasing concentration for 30 h. Maximum supersaturation was approx. 4.5 times the equilibrium solubility.</td>
<td>NA</td>
<td>Gao et al., 2013</td>
</tr>
<tr>
<td>Glibenclamide-serine 1:1</td>
<td>The co-amorphous systems provided a long-lasting (at least 24 h), two-fold supersaturation compared to the solubility of crystalline glibenclamide, in phosphate buffer. The extent of supersaturation was similar with amorphous drug alone, but it was achieved later. In biorelevant conditions, supersaturation dissolution was compared to the corresponding physical mixtures. The amino acids were not found to solubilize the drug in crystalline state and co-amorphous mixtures were not found to give better biorelevant solubility values compared to amorphous glibenclamide. Thus the amino acids may not act as precipitation inhibitors</td>
<td>Dissolution of the co-amorphous mixtures was compared to the corresponding physical mixtures. The amino acids were not found to solubilize the drug in crystalline state and co-amorphous mixtures were not found to give better biorelevant solubility values compared to amorphous glibenclamide. Thus the amino acids may not act as precipitation inhibitors</td>
<td>Heikkinen et al., 2015</td>
</tr>
<tr>
<td>Compound Combination</td>
<td>Description</td>
<td>Measurements/Results</td>
<td>References</td>
</tr>
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<tr>
<td>Simvastatin-lysine 1:1</td>
<td>Led to an increase in AUC$_{0-8h}$ compared to crystalline glibenclamide in FaSSIF (also with amorphous drug alone).</td>
<td>Lysine was not able to improve the drug dissolution when physically mixed with the drug. Supersaturation may thus be due to amorphization and drug–amino acid interactions, since it was not observed with amorphous drug alone.</td>
<td>Heikkinen et al., 2015</td>
</tr>
<tr>
<td>Simvastatin-glipizide 2:1, 1:1, 1:2</td>
<td>The system produced a long-lasting (8 h), twofold supersaturated state compared to the solubility of crystalline simvastatin in phosphate buffer. In FaSSIF, supersaturation led to the AUC$_{0-8h}$ significantly higher than that of pure crystalline and amorphous drug alone. In FeSSIF this was not observed.</td>
<td>NA</td>
<td>Löbmann et al., 2011b</td>
</tr>
<tr>
<td>Indomethacin-arginine 1:1</td>
<td>Co-amorphous formulations showed supersaturated concentrations of glipizide in phosphate buffer at 2h, but statistical significance could not be proved against crystalline drug or corresponding amorphous physical mixtures.</td>
<td>Precipitation studies in biorelevant media (solvent shift, DS 4) revealed that based on EGFs arginine had a statistically significant ability to inhibit indomethacin precipitation at low pH (FaSSIF blank and FaSSIF). Indomethacin-arginine salt formation, interaction between the guanidinium group of arginine and the aromatic groups of indomethacin and an arginine-dependent in situ amorphization of indomethacin were proposed crystallization inhibition mechanisms.</td>
<td>Lenz et al., 2015; Ojarinta et al., 2017</td>
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<tr>
<td>Indomethacin-phenylalanine 1:1</td>
<td>When powder dissolution was investigated in biorelevant media (FaSSIF, FeSSIF and blanks) media, clear indomethacin supersaturation in FaSSIF blank and FaSSIF was found. In FeSSIF blank and FeSSIF, the effect was less obvious. The highest excipient gain factor (EGF) -values for the co-amorphous form – were found in FaSSIF blank (~3.4) and FaSSIF (~10.7). An approx. four-fold supersaturation was observed from a tablet containing spray-dried co-amorphous indomethacin-arginine in phosphate buffer. This supersaturation was also observed with plain indomethacin-arginine powder, followed by faster recrystallization.</td>
<td>Precipitation studies (solvent shift, DS 4) revealed no precipitation inhibition by</td>
<td>Ojarinta et al., 2017</td>
</tr>
<tr>
<td>Indomethacin-tryptophan 1:1</td>
<td>blanks), a statistically significant slight improvement in the AUCs of degree of supersaturation (DS) -time profiles in every dissolution media were found when compared to amorphous indomethacin. EGF-values &lt;2 in all media. Indomethacin and ibuprofen showed high supersaturation (up to over 10-fold) in water with $C_{\text{max}}$ when α-maltose or palatinose were used as the sugar. With gliclazide and nifedipine supersaturation was modest (up to approx. fourfold), i.e. the model drug with a lower water solubility exhibited a greater extent of supersaturation. The co-amorphous loratadine-citric acid systems improved the dissolution of loratadine in water significantly (supersaturated conditions), i.e. for 1:1 system approx. 50-fold over crystalline loratadine and approx. 30-fold over amorphous loratadine after 4h. With 3:1 and 2:1 mixtures, the improvement was only modest. i.e. approx. threefold against crystalline loratadine.</td>
<td>phenylalanine in any media. Weak specific interactions between the components probably explain the inefficiency of phenylalanine to stabilize indomethacin supersaturation.</td>
<td>NA</td>
</tr>
<tr>
<td>Indomethacin, ibuprofen, gliclazide and nifedipine with trehalose, maltitol, α-maltose, and palatinose at 1% w/w drug loading</td>
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<tr>
<td>Loratadine-citric acid 3:1, 2:1 and 1:1</td>
<td>Since the co-amorphous loratadine-citric acid 1:1 showed a dissolution advantage over amorphous loratadine, it was suggested that hydrogen bonding between loratadine and citric acid (observed in the solid-state) may have inhibited the occurrence of nucleation and crystal growth, and prevented the recrystallization of loratadine. This was also reflected in the pharmacokinetic study in rats, as co-amorphous loratadine-citric acid 1:1 significantly improved absorption and bioavailability of loratadine over that of the crystalline form.</td>
<td></td>
<td>Wang et al., 2017</td>
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</table>

NA=not assessed
Figure legends

Fig. 1. Schematic showing a hypothetical dissolution profile of a crystalline drug and its amorphous counterpart with fast increase of drug concentration above the equilibrium solubility of the respective crystalline form (spring), and relatively fast formation of crystal nuclei, precipitation and drug concentrations reverting back to the level of crystalline solubility. For the spring and parachute case, a precipitation inhibitor effectively delays nucleation and crystal growth and allows the supersaturated state to prevail for longer. If the kinetics of crystallization are slow relative to the dissolution process, the drug concentration can exceed its amorphous solubility and undergo a liquid-liquid phase separation and subsequent crystallization.
Fig. 2. Scanning electron micrographs of the colloidal phase of ritonavir formed upon the dissolution of a ritonavir/PVP amorphous solid dispersion at time points of a) 5 min; b) 30 min; c) 60 min; d) 90 min; e) 120 min and f) 480 min. Reprinted with permission from Ilevbare, G.A., Liu, H., Pereira, J., Edgar, K.J., Taylor, L.S., 2013b. Influence of additives on the properties of nanodroplets formed in highly supersaturated aqueous solutions of ritonavir. Mol. Pharm. 10, 3392-3403. Copyright 2013 American Chemical Society.
**Fig. 3.** Concentration–time profiles with the magnification for the first 6 h (mean ± standard deviation) of co-amorphous indomethacin-arginine (ARG-IND), indomethacin-phenylalanine (PHE-IND), indomethacin-tryptophan (TRP-IND) and amorphous indomethacin (IND Amorph) in A FeSSIF blank, B FaSSIF blank, C FeSSIF, and D FaSSIF. The degree of supersaturation is provided in the vertical axis on the right. Reprinted with permission from Ojarinta, R., Heikkinen, A.T., Sievänen, E., Laitinen, R., 2017. Dissolution behavior of co-amorphous amino acid-indomethacin mixtures: The ability of amino acids to stabilize the supersaturated state of indomethacin. Eur. J. Pharm. Biopharm. 112, 85-95. Copyright 2017 Elsevier.
Fig. 4. Degree of supersaturation (DS, mean ± standard deviation)-time profiles of plain indomethacin (IND) and of IND in the presence of arginine (ARG), phenylalanine (PHE) or tryptophan (TRP) obtained from precipitation tests performed in A FeSSIF blank, B FaSSIF blank, C FeSSIF, and D FaSSIF. Reprinted with permission from Ojarinta, R., Heikkinen, A.T., Sievänen, E., Laitinen, R., 2017. Dissolution behavior of co-amorphous amino acid-indomethacin mixtures: The ability of amino acids to stabilize the supersaturated state of indomethacin. Eur. J. Pharm. Biopharm. 112, 85-95. Copyright 2017 Elsevier.