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Dissolution properties of co-amorphous drug-amino acid formulations in buffer and biorelevant media

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Co-amorphous formulations, particularly binary drug-amino acid mixtures, have been shown to provide enhanced dissolution for poorly-soluble drugs and improved physical stability of the amorphous state. However, to date the dissolution properties (mainly intrinsic dissolution rate) of the co-amorphous formulations have been tested only in buffers and their supersaturation ability remain unexplored. Consequently, dissolution studies in simulated intestinal fluids need to be conducted in order to better evaluate the potential of these systems in increasing the oral bioavailability of biopharmaceutics classification system class II drugs. In this study, solubility and dissolution properties of the co-amorphous simvastatin-lysine, gibenclamide-serine, glibenclamide-threonine and glibenclamide-serine-threonine were studied in phosphate buffer pH 7.2 and biorelevant media (fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF, respectively)). The co-amorphous formulations were found to provide a long-lasting supersaturation and improve the dissolution of the drugs compared to the crystalline and amorphous drugs alone in buffer. Similar improvement, but in lesser extent, was observed in biorelevant media suggesting that a dissolution advantage observed in aqueous buffers may overestimate the advantage *in vivo*. However, the results show that, in addition to stability advantage shown earlier, co-amorphous drug-amino acid formulations provide dissolution advantage over crystalline drugs in both aqueous and biorelevant conditions.

1. Introduction

Currently, the majority of drug molecules in discovery pipelines exhibit poor aqueous solubility which leads to poor bioavailability of these compounds and complicates their formulation as oral dosage forms (Williams et al. 2013). One strategy to overcome poor solubility is to utilize solubilizing additives to increase the thermodynamic solubility of the drug in the intestinal fluids. However, it has been proposed that solubilizers may not be effective in improving absorption if a decrease in effective permeability off-sets the increase in solubility (Miller et al. 2012). Therefore, supersaturating drug delivery systems which increase the intraluminal drug concentration above the thermodynamic solubility of the compound in the intestinal fluids is a promising approach for overcoming poor solubility (Alonzo et al. 2010; Brouwers et al. 2009; Ozaki et al. 2012). Transformation of drugs into amorphous forms is one of the key techniques to induce supersaturation in intestinal fluids and thus improve the bioavailability of drugs since the high-energy amorphous form possesses a higher apparent solubility (supersaturation) and dissolution rate compared to the crystalline state (Kaushal et al. 2004; Yu 2001). The major challenge when using amorphous drug formulations however is their low physical stability both during storage and upon dissolution (Alonzo et al. 2010; Newman et al. 2012). Poor stability during storage limits the shelf life of amorphous formulations. Moreover, poor stability and transformation to a crystalline form upon dissolution leads to an inability to induce a supersaturated state. Furthermore, in order to improve oral bioavailability, the supersaturated state must be maintained over a sufficient time period to allow drug absorption through the intestinal wall (Alonzo et al. 2012; Brouwers et al. 2009; Friesen et al. 2008). If a drug and an excipient dissolve rapidly to yield a supersaturated state (spring), supersaturation can be maintained for example by the dissolved excipient inhibition of solution-mediated nucleation and crystalline growth of the drug (parachute) (Augustijns and Brewster 2012; Guzman et al. 2007). Some polymer excipients for example, have been shown to be able to maintain a supersaturated state for drugs (Curatolo et al. 2009). However, if the rate of crystallization of the amorphous material is fast upon contact with the dissolution medium, it will not be able to generate a concentration higher than that produced by the crystalline material (Alonzo et al. 2010). In addition, bile salt micelles and other lipids found inherently in the GI tract may also function as solubilizers or nucleation or crystal growth inhibitors to maintain high levels of supersaturation (Mithani et al. 1996). Thus for obtaining long-term supersaturation upon *in vivo* release, the tendency for the drug to remain in an amorphous form associated to colloidal structures of the excipient (e.g. polymers), with surfactants or with natural surfactant-like materials found in the dissolution medium (GI tract liquids), is generally essential (Newman et al. 2012).

Drug dissolution properties of amorphous formulations are often evaluated in aqueous buffers rather than intestinal fluids or biorelevant media which simulate intestinal fluids. However, both dissolution rate and solubility may be drastically different in intestinal fluids compared to aqueous buffers (Fagerberg et al. 2010). Lipophilic drugs partition to bile salt micelles present in the intestinal fluids, which increases the apparent solubility. Improved solubility increases the concentration gradient between particle surface and bulk phase which drives the dissolution. However, at the same time, partition into bile salt micelles limits the diffusion coefficient of the drug, thus potentially limiting the dissolution rate (Okazaki et al. 2008). Therefore, formulation behavior observed in aqueous buffers (improvement in dissolution rate and formation of supersaturated concentrations) does not necessarily reflect the drug behavior in the presence of constituents of the intestinal fluids. Consequently, *in vitro* studies in various simulated intestinal fluids should be conducted for amorphous formulations.

Recently, co-amorphous drug mixtures with low molecular weight excipients have been shown to be a promising approach for dissolution improvement of poorly soluble drugs and stabilization of the amorphous state (Allesø et al. 2009; Chieng et al. 2009; Löbmann et al. 2011, 2012, 2013a, b, c). In particular, drug combinations with amino acids (AAs) have the potential to serve as a new platform technique to overcome challenges associated with an amorphous form of poorly soluble drugs (Löbmann et al. 2013b, c). In a recent study, we have investigated co-amorphous formulations of the drugs simvastatin (SVS) and glibenclamide (GBC) with AAs (Laitinen et al. 2014). From the different AAs used, SVS formed a co-amorphous mixture only with the AA lysine (LYS) which is present at the SVS binding site in the drug receptor. GBC formed co-amorphous binary mixtures with its receptor AA serine (SER) and the non-receptor AA threonine (THR) in addition to a ternary combination containing both AAs. A single glass-transition (T_g) was obtained for the mixtures showing that homogenous amorphous blends were formed. The formation of co-amorphous mixtures provided a physical stability advantage over the amorphous drugs alone. SVS-LYS was found to be stable for three months when stored at 40 °C/0% RH whereas GBC-SER was found to be stable for six months in these conditions.

In the current study, solubility and dissolution properties of the abovementioned co-amorphous mixtures, i.e. SVS-LYS, GBC-SER, GBC-THR and GBC-SER-THR were examined in phosphate buffer pH 7.2 and biorelevant media (fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF)). Biorelevant media were used in order to investigate possible supersaturation of the drugs in simulated intestinal fluids during biologically relevant time frames. This is essential for considering a possible bioavailability advantage provided by an amorphous formulation (Newman et al. 2012). Despite this fact, biorelevant solubility studies have not been reported very often for amorphous formulations (e.g. solid dispersions) (Newman et al. 2012). Especially, they have not been conducted previously for co-amorphous systems for which the dissolution studies so far have been conducted mainly by intrinsic methods, thus there is no information on the supersaturation ability of these systems in aqueous solutions (Allesø et al. 2009; Löbmann et al. 2011; 2013b).

2. Investigations, results and discussion

The equilibrium solubility in pH 7.2 buffer for the crystalline SVS and the apparent solubility for the amorphous SVS were found to be 0.3 ± 0.2 μg/ml and 0.1 ± 0.1 μg/ml, respectively, which were not statistically different. Thus, converting the crystalline form of the drug into an amorphous form did not improve the solubility of SVS which has also been observed previously (Löbmann et al. 2012). This may be due to recrystallization of the amorphous state during the solubility test (72 h) and/or wetting problems of the amorphous SVS-powder (see chapter 2.1). Values in FaSSIF (pH 6.5) and FeSSIF (pH 5.0) blank buffers could not be obtained (below detection limit). For GBC, the crystalline and amorphous solubilities in pH 7.2 were found to be 9.6 ± 0.4 μg/ml and 43.6 ± 5.1 μg/ml, respectively. The apparent solubility for amorphous GBC was statistically significantly higher than for crystalline GBC. Solubility values could not be obtained for GBC in FeSSIF blank buffer. In FaSSIF blank buffer the solubility for crystalline and amorphous GBC was found to be 1.5 ± 0.2 μg/ml and 4.0 ± 0.8 μg/ml, respectively. Since the solubilities for both drugs in the crystalline and amorphous state were highest in pH 7.2 buffer, the powder dissolution test in buffer was conducted in this medium, in order to obtain comparable results.

2.1. Dissolution in buffer solution

In the powder dissolution studies it was observed, that the dissolution profiles of crystalline SVS and SVS from SVS-LYS PM were similar. Furthermore, the dissolution rate of amorphous SVS was even lower than that of crystalline SVS, since no detectable amount of SVS was dissolved during the first 8 h (Fig. 1a). This is probably due the tendency of amorphous SVS to form a clump when introduced to dissolution media instead of spreading as dispersed particles in the dissolution medium. In contrast, such clumping behavior was not observed with the co-amorphous SVS-LYS, and this system was able to increase the dissolution rate of SVS and produce concentrations approximately twice the equilibrium solubility of SVS. The supersaturated state prevailed for at least 8 h and SVS dissolution rate was found to be statistically significantly enhanced ($p < 0.05$) compared to crystalline SVS (between 40 min and 8 h). This means that LYS might be able to act as a co-amorphous excipient that improves the wetting properties and promotes supersaturation of SVS. Interestingly, LYS was not able to improve the SVS dissolution when physically mixed with the drug. In the case of GBC, the dissolution profiles of crystalline GBC and the PMs of GBC-SER, GBC-THR and GBC-SER-THR were found to be virtually identical (Fig. 1b). In contrast, a substantial (statistically significant) increase in dissolution rate was observed with all the amorphous formulations of GBC when compared to crystalline GBC. All systems were able to produce concentrations approximately two times higher than the equilibrium solubility of GBC. In the presence of AAs this concentration was reached within 6 min while 30 min were necessary in the absence of AAs (Fig. 1b, magnification). Thus, all the co-amorphous formulations dissolved statistically significantly faster compared to amorphous drug alone, but there was no difference between the formulations with different AAs. Thereby, a spring-effect by the AAs was observed. It is again interesting to note that the AAs were not able to improve the dissolution when only physically mixed with the drug. With all these amorphous samples, the supersaturated state lasted for the whole duration of the dissolution test, with no sign of reverting back towards the equilibrium solubility of crystalline GBC. As indicated by the solubility test (pH 7.2), the supersaturated state of amorphous GBC can be considered relatively stable, i.e. it can be expected to last at least for 72 h. Furthermore, the supersaturation can be regarded as 'true' (i.e. increase in molecularly dissolved, free drug) occurred as the level of supersaturation was similar in amorphous GBC alone and co-amorphous formulations (i.e. no effect of excipient) and the dissolution medium did not contain any solubilizing agents (Buckley et al. 2013).

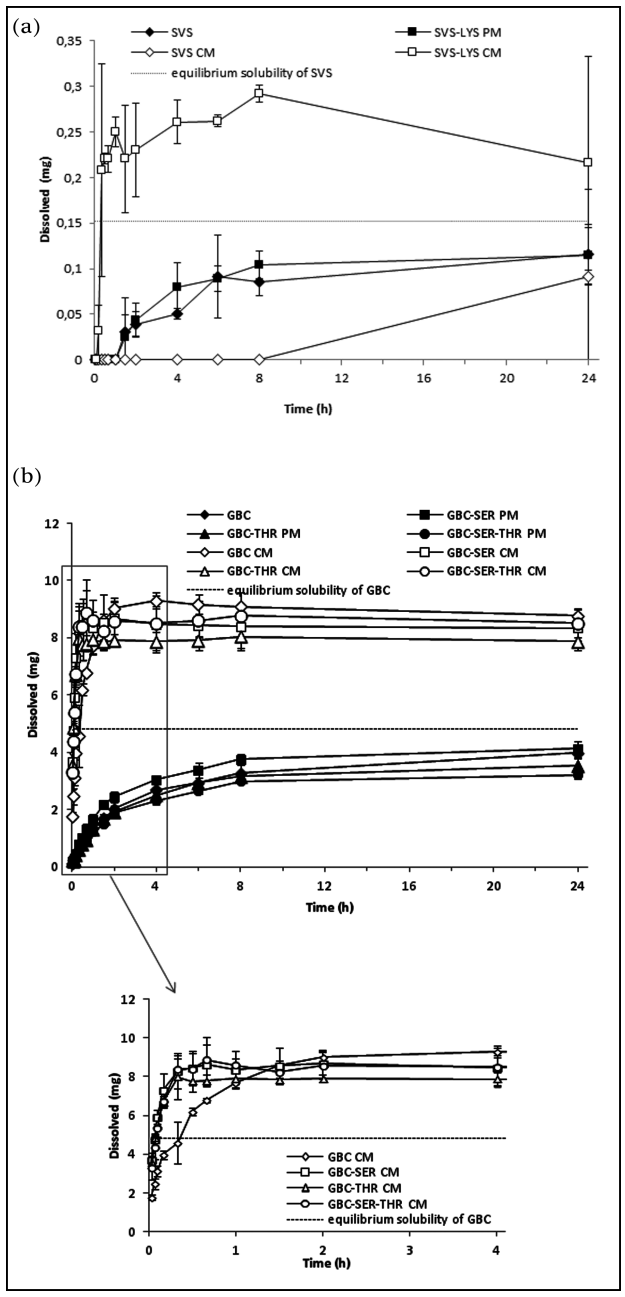

Fig. 1: Powder dissolution profiles at pH 7.2 up to 24 h (n = 3) for a) crystalline and amorphous simvastatin (SVS and SVS CM, respectively) and simvastatin-lysine (SVS-LYS) physical (PM) and co-amorphous mixtures (CM) and b) crystalline and amorphous glibenclamide (GBC and GBC CM, respectively), glibenclamide-serine (GBC-SER), glibenclamide-threonine (GBC-THR) and GBC-SER-THR PM and CM mixtures showing also a magnification of the dissolution profiles of GBC CM, GBC-SER CM, GBC-THR CM and GBC-SER-THR CM during the first 90 minutes of the test.

The AAs improved the dissolution only when present in the co-amorphous mixture. All the AAs used are soluble in water (SER 5.023 g/100 ml, i.e soluble, and LYS and THR very soluble in water (CRC Handbook of Chemistry and Physics 1982; The Merck Index 1996) and thus they may improve the wetting properties of the hydrophobic drugs but this effect was not seen in the physical mixtures. In the case of SVS, the hydrophilic LYS was able to improve the wetting properties of the amorphous SVS by preventing the formation of a poorly soluble clump, which led to an improved dissolution rate. In the co-amorphous mixtures, weak interactions exist between the drug and AA (Laitinen et al. 2014) which may explain the improved drug dissolution in the case of co-amorphous mixtures.

2.2. Dissolution in biorelevant media

Drug dissolution in the biorelevant media is shown in Fig. 2a-d. In addition, $AUC_{0\text{-}8h}$ (area under curve, from 0 to 8 h) values for each sample were calculated in order to obtain the total concentration dissolved ($\mu g\ ml^{-1}\ h$) during the solubility test and these are shown in Fig. 3. The bile salt concentration effect was obvious for SVS, as it was observed that FaSSIF and FeSSIF media were able to solubilize SVS (Figs. 2a and b). Concentrations were approximately three times higher in FeSSIF (bile salt concentration 15 mM) compared to FaSSIF (bile salt concentration 3 mM). For lipophilic compounds it is known that solubility increases with increasing bile salt concentration of the medium when the system is above the critical micelle concentration (CMC, 0.6 mM for mixed taurocholate-lecitin micelles at 4:1 concentration ratio (Glomme et al. 2006)), i.e. from 3 mM in the fasted state to 15 mM (Mithani et al. 1996). Similarly, an increase in the $AUC_{0\text{-}8h}$ values with increasing bile salt concentration was observed. $AUC_{0\text{-}8h}$ values were four times higher in FeSSIF compared to those obtained from FaSSIF (Fig. 3). However, there was no statistically significant difference found among the $AUC_{0\text{-}8h}$ of the different formulations in FeSSIF, whereas in FaSSIF the $AUC_{0\text{-}8h}$ of the co-amorphous SVS-LYS mixture was found to be significantly higher than those of pure crystalline and amorphous SVS. The absence of solubility differences in FeSSIF was probably due to the large solubilizing effect of the high concentration of bile salts overruling the solubility improvement by amorphisation. This suggests that two solubility enhancing factors exist for SVS in the biorelevant media, i.e. formation of the co-amorphous mixture with LYS and the solubilizing effect of micelle concentration of the medium. Since SVS is a neutral compound, hydrophobic and lipophilic interactions with the media components are mainly responsible for the solubility enhancement in biorelevant media and the pH effect can be ruled out (Ottaviani et al. 2010). Amorphization became thus insignificant as dissolution improving mechanism when the micelle concentration was high (FeSSIF).

In the case of GBC, the concentration of dissolved crystalline GBC was not found to be statistically significantly different from that from the PMs in both biorelevant media (Fig. 2c and d). Thus, the AAs were not able to enhance the solubility of GBC when prepared as physical mixtures. Instead, amorphization in general caused a substantial increase in total drug concentration dissolved in these media since all amorphous GBC samples provided a statistically significant increase in concentration of dissolved drug compared to the their crystalline counterpart (except GBC CM and GBC-SER-THR CM in FeSSIF at 4 h and GBC-SER-THR CM in FaSSIF and GBC-SER in FeSSIF at 48 h). However, the co-amorphous mixtures did not show a statistically significant difference compared to pure amorphous GBC and none of the co-amorphous mixtures was found to be better than the others (Fig. 2c and 2d). The GBC $AUC_{0\text{-}8h}$ values were in general higher in FaSSIF compared to FeSSIF (except for the PMs (the reason for this is currently unclear) Fig. 3). This can be explained by two opposite effects. Due to the acidic nature of GBC (pK_a 5.1 (Avdeef 2007)) a pH change from 5.0 to 6.5 (from FeSSIF to FaSSIF) is contributing towards higher solubility (ionization of GBC). In contrast, the decrease in bile salt concentration from 15 mM to 3 mM is contributing towards lower solubility. The impact of bile salt micelles on ionized GBC is probably negligible compared to the increase in solubility of unionized GBC (Sugano 2009). In these experimental pHs, there is always both ionized and neutral GBC present, and the solubility of GBC in presence of bile salts is always higher than in blank buffer at the corresponding pH (as seen when comparing the solubility values between FaSSIF blank ($1.5 \pm 0.2\ \mu g/ml$) and FaSSIF ($2.0 \pm 0.3\ \mu g/ml$, Fig. 2c). Therefore, the overall

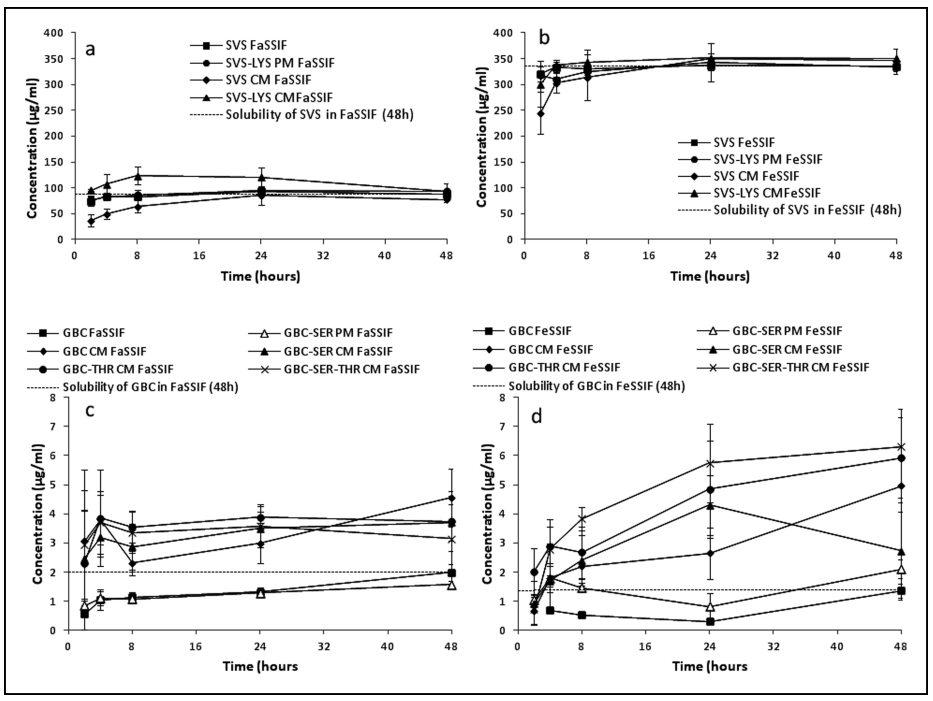

Fig. 2: Dissolved drug concentrations (mean µg/ml ± sd, n = 3) in biorelevant media as a function of time for a) crystalline and amorphous simvastatin (SVS and SVS CM) and simvastatin-lysine (SVSLYS) physical (PM) and co-amorphous (CM) mixtures in fasted state simulated intestinal fluid (FaSSIF); b) crystalline and amorphous simvastatin (SVS and SVS CM) and SVS-LYS PM and CM in fed state simulated intestinal fluid (FeSSIF); c) crystalline and amorphous glibenclamide (GBC and GBC CM, respectively), glibenclamide-serine (GBC-SER) PM as an example of the PMs and GBC-SER, glibenclamide-threonine (GBC-THR) and GBC-SER-THR CM in FaSSIF and d) crystalline and amorphous glibenclamide (GBC and GBC CM, repectively), GBC-SER PM as an example of the PMs and GBC-SER, GBC-THR and GBC-SER-THR CM in FeSSIF.

change in solubility is dependent on relative contributions of a pH effect and a bile salt micelle effect. In the case of GBC the pH effect is more significant than the bile salt effect and thus, a higher solubility and $AUC_{0\text{-}8h}$ in FaSSIF than in FeSSIF is seen, as also shown in other studies (Löbenberg et al. 2000). Looking at the $AUC_{0\text{-}8h}$ values it can be concluded, that all the amorphous formulations provided a significant solubility advantage over the crystalline GBC, but the co-amorphous mixtures were not found to give better biorelevant solubility values compared to amorphous GBC (Fig. 3).

The experimental variability in the dissolution profiles of some of the formulations (especially amorphous) was found to be large. This can be explained by the solvent-mediated recrystallization phenomenon occurring at different rates in different samples. Nevertheless, a statistically significant difference between the co-amorphous samples and their crystalline counterparts could be established. In addition, it has been observed that differences in hydrodynamic conditions may cause differences in the recrystallization of the amorphous supersaturated state. Thus, the increased kinetic energy resulting from

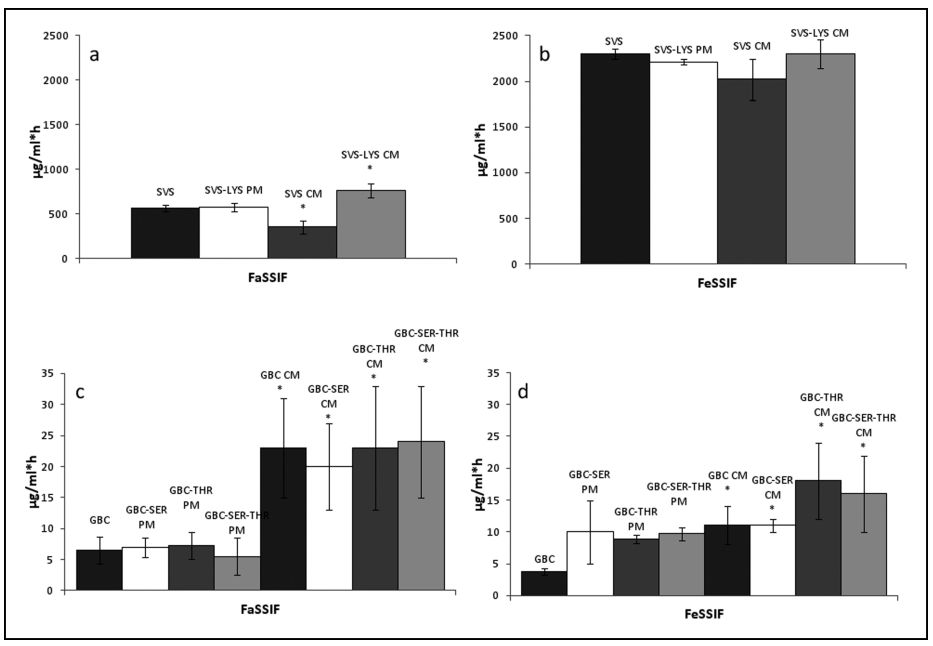

Fig. 3: Area under curve ($AUC_{0-8\,h}$, µg/ml h) mean values ± sd (n = 3) calculated from the concentrations of simvastatin (SVS) and glibenclamide (GBC) in fasted state simulated intestinal fluid (FaSSIF) and fed state intestinal fluid (FeSSIF) for a) SVS formulations in FaSSIF; b) SVS formulations in FeSSIF; c) GBC formulations in FaSSIF and d) GBC formulations in FeSSIF. Formulations with the AUC statistically significantly different compared to the corresponding crystalline drug are marked with an asterisk.

extensive mixing may assist in overcoming the activation hurdle for nuclei formation (Bevernage et al. 2013; Brouwers et al. 2009; Lindfors et al. 2008). This can have implications on the recrystallization rates in the different experimental setups and thus also on the experimental variation. It has been reported that the precipitation rate of a basic BCS class II model drug was remarkably slower in the shaking model compared to the stirring (paddle) model (Carlert et al. 2010).

The earlier time points of the biorelevant dissolution test (4 h) are more relevant for the intestinal transit time and may, therefore, better reflect the drug solubilization *in vivo* (Schwebel et al. 2011). In the case of SVS, the drug reached and exceeded its equilibrium solubility comparatively fast (at 4 h) from SVS-LYS co-amorphous formulation in FaSSIF and FeSSIF, respectively. In the case of GBC, such a supersaturation phenomenon was observed with all co-amorphous formulations at 4 h. This supersaturation of amorphous formulations might offer a bioavailability advantage over the crystalline drugs (Newman et al. 2012). However, it has been hypothesized that a positive effect on GI-tract membrane permeation would occur only if a formulation induces an increased concentration of molecularly dissolved drug (i.e. "true" supersaturation). In contrast, any apparent solubility enhancement, which reflects an increase in solubility evoked by solubilizing agents (in the formulation or in the dissolution medium) is regarded less likely to enhance permeability (Buckley et al. 2013). This study has shown that at least for amorphous GBC the supersaturation could be called "true" since it occurs whether or not GBC is formulated with excipients or regardless of the lack or presence of solubilizers in the dissolution medium. In the biorelevant media, supersaturation was less evident and the differences in the dissolution properties between the formulations were ambiguous. Thus regarding dissolution, amorphous formulation seems beneficial for both model drugs used whereas there is no unambiguous advantage of formulating these model drugs as co-amorphous formulations. Furthermore, the results show that dissolution advantage of amorphous formulation observed in aqueous buffers may not reflect the dissolution in the presence of bile acids, and exemplify the importance of evaluation of the possible dissolution and bioavailability advantage of amorphous formulations in biorelevant conditions. Nonetheless, with an improved storage stability observed in earlier studies, co-amorphous drug-AA mixtures can be beneficial for formulation of solid dosage forms (Laitinen et al. 2014).

3. Experimental

3.1. Materials

Simvastatin (SVS, 418.6 g/mol, USP grade), glibenclamide (GBC, 494.0 g/mol, USP grade), L-lysine (LYS, 146.2 g/mol, medicine grade), L-serine (SER, 105.1 g/mol, medicine grade) and L-threonine (THR, 119.1 g/mol, medicine grade) were all purchased from Hangzhou Dayangchem CO Ltd (Hangzhou City, China). All compounds were used as received. Commercially available SIF Powder® (Phares AG, Muttenz, Switzerland) was used for preparing the FaSSIF and FeSSIF solutions in the blank buffers according to the manufacturer's protocol (SIF Powder Original Info, 2014).

3.2. Methods

3.2.1. Preparation of amorphous materials

Co-amorphous blends were prepared by cryomilling (CM) at 30 Hz for 60 min in an oscillatory ball mill (Mixer Mill MM400, Retch GmbH & Co., Haan, Germany). A total mass of 500 mg of the crystalline compounds, or the appropriate amount of drug and AA(s) at the molar ratios 1:1 (drug:AA) or 1:1:1 (drug:AA1:AA2), respectively, was placed into 25 ml milling jars with two 15 mm stainless steel balls. The milling jars were immersed in liquid nitrogen for 2 min prior to and during milling (at 10 min intervals).

3.2.2. Solubility and dissolution testing

Equilibrium solubilities of crystalline SVS and GBC and the apparent solubilities of their amorphous counterparts were measured at 37 °C. An excess amount of the drug was placed into the dissolution vessel with 300 ml of USP phosphate buffer (pH 7.2) or the blank buffers of fasted state simulated intestinal fluid (FaSSIF blank, buffered using sodium dihydrogenphosphate, pH 6.5) and fed state simulated intestinal fluid (FeSSIF blank, buffered using acetic acid, pH 5). The suspensions were mixed with a paddle (100 rpm) for 72 h and then filtered through a 0.2 μm membrane filter and analyzed with HPLC as described below. The experiments were conducted in triplicate.

The powder dissolution profiles over a period of 24 h were measured using the USP paddle method (Distek 2100C Dissolution system, Distek, North Brunswick, NJ, USA), with a rotation speed of 50 rpm and 500 ml of USP phosphate buffer (pH 7.2) as a dissolution medium at 37 ± 0.5 °C. The amount of powder (equivalent to 20 mg the drug) was weighed and transferred to the bottom of a dissolution vessel, thus based on the solubility test results, non-sink conditions prevailed during the test. It is important to note that only non-sink conditions in the dissolution medium allow adequate investigation of supersaturation, as suggested in a study where meaningful *in vitro–in vivo* correlations for silica based formulations and solid dispersions were only achieved using non-sink *in vitro* dissolution approaches (Augustijns and Brewster 2012; Bevernage et al. 2013). The preheated medium was poured onto the sample. Samples (5 ml) were withdrawn and immediately replaced with buffer. The samples were diluted after collection with acetonitrile (ACN) corresponding to the mobile phase composition (see below) and analyzed with HPLC as described below. The experiments were conducted in triplicate.

Dissolution testing in biorelevant media was conducted at 37 °C using FaSSIF and FeSSIF solutions (Galia et al. 1998; SIF Powder Original Info 2014). The testing was continued longer than the total human intestinal transit time, i.e. for 48 h, in order to reach the equilibrium solubility. According to the manufacturer, the solutions prepared from SIF Powder® need to be used within 48 h, thus the tests conducted in these media were stopped at that point. SIF Powder® contains taurocholate and lecithin in 4:1 molar ratio; the resulting FaSSIF solution contains 3 mM sodium taurocholate and the FeSSIF solution 15 mM sodium taurochlorate. An excess amount of the solid compound was added to small Erlenmeyer flasks containing 10 ml of the biorelevant medium which thereafter were placed in a preheated shaking incubator (OLS 200, Grant Instruments, Cambridge, England). The temperature was set to 37 °C and the utilized shaking rate was 100 rpm. Samples with a volume of 1 ml were taken at predetermined time points (2, 4, 8, 24 and 48 h), filtered through a 0.2 μm pore size filter and thereafter diluted with ACN corresponding to the mobile phase composition (see below). Samples were further diluted (with ACN/dissolution medium 70/30) if the concentrations exceeded the concentration of the highest standard. Sample aliquots of 500 μl were analyzed using HPLC as described below. The experiments were conducted in triplicate. The area under the curve (AUC) of a plot of the concentration of dissolved drug as a function of time was calculated using Origin Pro 7.5 SR4 by linear trapezoidal integration (OriginLab Corporation, Northampton, MA, USA). The area was calculated between 0 and 8 h ($AUC_{0\text{-}8h}$) considering the total human intestinal transit time (Thelen et al. 2011; Yu et al. 1996).

The drug concentrations were analyzed by HPLC. A Gilson HPLC system, consisting of a 234 Autoinjector (Gilson, Roissy-en-France, France), 321 Pump, UV/Vis detector, System interface module and Unipoint™ LC system version 3.01 software (all from Gilson, Middleton, WI, USA) was used with a Phenomenex Gemini-NX 5u C18 110A (250 × 4.60 mm) column. The mobile phase consisted of acetonitrile (ACN) + 0.1% trifluoro acetic acid (TFA) (70%) and H_2O and 0.1% TFA (30%) with a flow rate of 1.2 ml/min. The detection wavelength was 225 nm for GBC and 238 nm for SVS. Standard solutions (0.1, 0.5, 1, 5, 25, 50, 100 μg/ml for SVS and 0.5, 1, 5, 25, 50, 100 μg/ml for GBC) were prepared in ACN/H_2O 70/30. The resulting standard curves were linear in the concentration ranges mentioned above (r^2 was 0.9999 for both drugs) and the retention times were 9.3 ± 0.1 min for SVS and 3.9 ± 0.1 min for GBC. The repeatability of the method (RSD) was 2.3% for SVS (0.1 μg/ml) and 2.9% for GBC (0.5 μg/ml), respectively.

3.2.3. Data analysis

Data from solubility studies were compared by performing single-factor ANOVA analysis. The concentrations (μg/ml) at different time points and $AUC_{0\text{-}8h}$-values were compared to investigate if there were differences between the different mixtures. Differences were considered significant with p-values < 0.05 (95% confidence level).

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References

Allesø M, Chieng N, Rehder S, Rantanen J, Rades T, Aaltonen J (2009) Enhanced dissolution rate and synchronized release of drugs in binary systems through formulation: Amorphous naproxen-cimetidine mixtures prepared by mechanical activation. J Control Release 136: 45–53.

Alonzo DE, Zhang GGZ, Zhou D, Gao Y, Taylor LS (2010) Understanding behavior of amorphous pharmaceutical systems during dissolution. Pharm Res 27: 608–618.

Augustijns P, Brewster ME (2012) Supersaturating drug delivery systems: fast is not necessarily good enough. J Pharm Sci 101: 7–9.

Avdeef A (2007) Solubility of sparingly-soluble ionizable drugs, Adv. Drug Deliv. Rev. 59 568–590.

Bevernage J, Brouwers J, Brewster ME, Augustijns P (2013) Evaluation of gastrointestinal drug supersaturation and precipitation: Strategies and issues. Int J Pharm 453: 25–35.

Brouwers J, Brewster ME, Augustijns P (2009) Supersaturating drug delivery systems: Answer to solubility-limited oral bioavailability. J Pharm Sci 98: 2549–2572.

Buckley ST, Frank KJ, Fricker G, Brandl M (2013) Biopharmaceutical classification of poorly soluble drugs with respect to "enabling formulations". Eur J Pharm Sci 50: 8–16.

Carlert S, Pålsson A, Hanisch G, von Corswant C, Nilsson C, Lindfors L, Lennernäs H, Abrahamsson B (2010) Predicting intestinal precipitation—a case example for a basic BCS class II drug. Pharm Res 27: 2119–2130.

Chieng N, Aaltonen J, Saville D, Rades T (2009) Physical characterization and stability of amorphous indomethacin and ranitidine hydrochloride binary systems prepared by mechanical activation. Eur J Pharm Biopharm 71: 47–54.

CRC Handbook of Chemistry and Physics (1982) 63rd ed., CRC Press Inc., Boca Raton, pp. C-758.

Curatolo W, Nightingale JA, Herbig SM (2009) Utility of hydroxypropylmethylcellulose acetate succinate (HPMCAS) for initiation and maintenance of drug supersaturation in the GI milieu. Pharm Res 26: 1419–1431.

Fagerberg JH, Tsinman O, Sun N, Tsinman K, Avdeef A, Bergström CAS (2010) Dissolution rate and apparent solubility of poorly soluble drugs in biorelevant dissolution media. Mol Pharm 7: 1419–1430.

Friesen DT, Shanker R, Crew M, Smithey DT, Curatolo WJ, Nightingale JAS (2008) Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: An overview. Mol Pharm 5: 1003–1019.

Galia E, Nicolaides E, Hörter D, Löbenberg R, Reppas C, Dressman JB (1998) Evaluation of Various Dissolution Media for Predicting *In Vivo* Performance of Class I and II Drugs. Pharm Res 15: 698–705.

Glomme A, März J, Dressman JB (2006) Predicting the intestinal solubility of poorly soluble drugs. In: Testa B, Krämer SD, Wunderli-Allenspach H, Folkers G (eds.) Pharmacokinetic profiling in drug research: biological, physicochemical and computational strategies, Verlag Helvetica Chimica Acta, Switzerland, pp. 259–280.

Guzman HR, Tawa M, Zhang Z, Ratanabanangkoon P, Shaw P, Gardner CR, Chen H, Moreau J, Almarsson O, Remenar JF (2007) Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. J Pharm Sci 96: 2686 – 2702.

Kaushal AM, Gupta P, Bansal AK (2004) Amorphous drug delivery systems: Molecular aspects, design and performance. Crit Rev Ther Drug Carrier Syst 21: 133–193.

Laitinen R, Löbmann K, Grohganz H, Strachan C, Rades T (2014) Amino acids as co-amorphous excipients for simvastatin and glibenclamide: physical properties and stability. Mol Pharm 11: 2381–2389.

Lindfors L, Forssén S, Westergren J, Olsson U (2008) Nucleation and crystal growth in supersaturated solutions of a model drug. J Colloid Interface Sci 325: 404–413.

Löbenberg R, Krämer J, Shah VP, Amidon GL, Dressman JB (2000) Dissolution testing as a prognostic tool for oral drug absorption: Dissolution behavior of glibenclamide. Pharm Res 17: 439–444.

Löbmann K, Laitinen R, Grohganz H, Gordon KC, Strachan C, Rades T (2011) Co-amorphous drug systems: enhanced physical stability and dissolution rate of indomethacin and naproxen. Mol Pharm 8: 1919–1928.

Löbmann K, Strachan C, Grohganz H, Rades T, Korhonen O, Laitinen R (2012) Co-amorphous simvastatin and glipizide combinations show improved physical stability without evidence of intermolecular interactions. Eur J Pharm Biopharm 8: 159–169.

Löbmann K, Laitinen R, Grohganz H, Strachan C, Rades T, Gordon KC (2013a) A theoretical and spectroscopic study of co-amorphous naproxen and indomethacin. Int J Pharm 453: 80–87.

Löbmann K, Grohganz H, Laitinen R, Strachan C, Rades T (2013b) Amino acids as co-amorphous stabilizers for poorly water soluble drugs – Part 1: Preparation, stability and dissolution enhancement. Eur J Pharm Biopharm 85: 873–881.

Löbmann K, Laitinen R, Strachan C, Rades T, Grohganz H (2013c) Amino acids as co-amorphous stabilizers for poorly water-soluble drugs – Part 2: Molecular interactions. Eur J Pharm Biopharm 85: 882–888.

Miller JM, Beig A, Carr RA, Webster GK, Dahan A (2012) The solubility – permeability interplay when using cosolvents for solubilization: Revising the way we use solubility-enabling formulations. Mol Pharm 9: 581 – 590.

Mithani SD, Bakatselou V, TenHoor CN, Dressman JB (1996) Estimation of the increase in solubility of drugs as a function of bile salt concentration. Pharm Res 13: 163–167.

Newman A, Knipp G, Zografi G (2012) Assessing the performance of amorphous solid dispersions. J Pharm Sci 101: 1355–1377.

Okazaki A, Mano T, Sugano K (2008) Theoretical dissolution model of polydisperse drug particles in biorelevant media. J Pharm Sci 97: 1843–1852.

Ottaviani G, Gosling DJ, Patissier C, Rodde S, Zhou L, Faller B (2010) What is modulating solubility in simulated intestinal fluids? Eur J Pharm Sci 41: 452–457.

Ozaki S, Minamisono T, Yamashita T, Kato T, Kushida I (2012) Supersaturation–nucleation behavior of poorly soluble drugs and its impact on the oral absorption of drugs in thermodynamically high-energy forms. J Pharm Sci 101: 214–222.

Schwebel HJ, van Hoogevest P, Leigh MLS, Kuentz M (2011) The apparent solubilizing capacity of simulated intestinal fluids for poorly water-soluble drugs. Pharm Dev Tech 16: 278–286.

SIF Powder Original Info, www.biorelevant.com

Sugano K (2009) Computational oral absorption simulation for low-solubility compounds. Chem Biodiv 6: 2014–2029.

The Merck Index (1996), Merck&Co. Inc., Whitehouse Station.

Thelen K, Coboeken K, Willmann S, Burghaus R, Dressman JB, Lippert J (2011) Evolution of a detailed physiological model to simulate the gastrointestinal transit and absorption process in humans, Part 1: Oral solutions. J Pharm Sci 100: 5324–5345.

Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, Porter CJH (2013) Strategies to address low drug solubility in discovery and development. Pharmacol Rev 65: 315–499.

Yu L (2001) Amorphous pharmaceutical solids; preparation, characterization and stabilization. Adv Drug Deliv Rev 48: 27–42.

Yu LX, Crison JR, Amidon GL (1996) Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. Int J Pharm 140: 111–118.