Biphasic drug release testing coupled with diffusing wave spectroscopy for mechanistic understanding of solid dispersion performance

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Abstract

Amorphous Solid dispersions (ASDs) represent an important formulation technique to achieve supersaturation in gastrointestinal fluids and to enhance absorption of poorly water-soluble drugs. Drug release from such systems is complex due to emergence of different colloidal structures and potential drug precipitation, which can occur in parallel to absorption. The latter drug uptake from the intestinal lumen can be simulated by an organic layer in a biphasic *in vitro* test, which was employed in this work to mechanistically study the release of ketoconazole from ASDs produced by hot melt extrusion using different HPMCAS grades. A particular aim was to introduce diffusing wave spectroscopy (DWS) to biopharmaceutical testing of solid dispersions. Results indicated that amorphous formulations prevented crystallization of the weakly basic drug upon transfer into the intestinal medium. Microrheological differences among polymer grades and plasticizers were revealed in the aqueous phase, which affected drug release and subsequently uptake into the organic layer. The results indicate that DWS can be employed as a new non-invasive tool to better understand drug release from solid dispersions. This novel light scattering technique is highly promising for future biopharmaceutical research on supersaturating systems such as solid dispersions.

1. Introduction

Amorphous solid dispersions (ASDs) are one of the most widely employed methods to formulate poorly water-soluble drugs. To increase the apparent solubility and/or dissolution rate of a poorly soluble compound, it is encouraged to consider the amorphous form of a drug. The compound in an amorphous high-energy state is usually formulated in a polymer matrix system, which should be kinetically stabilized throughout the targeted shelf-life of the product. (Newman, 2015) Thus, polymers should have a stabilizing function in the formulation to prevent drug crystallization in solid dispersions (Baghel et al., 2016; Chiou and Riegelman, 1971a, 1971b; Leuner, 2000; Serajuddin, 1999; Serajuddln, 1999) by means of specific interactions with the active compound and via a general reduction of molecular mobility. (Khougaz and Clas, 2000; Tantishaiyakul et al., 1996; Taylor and Zografi, 1997) A large number of polymers employed for solid dispersion are water soluble in all pH conditions, but there are also enteric polymers that contain acidic groups that become ionized at higher pH to facilitate swelling and some water solubility. The latter group of polymers includes hydroxyl propyl methylcellulose acetate-succinate (HPMCAS) and methacrylate-based enteric coating polymer systems, such as the Eudragits. (Newman, 2015; Warren et al., 2010)

Biopharmaceutical classification system (BCS) class II drugs are characterized by low solubility and high permeability. (Amidon et al., 1995; Buckley et al., 2013, 2012) In addition, weakly basic drugs have higher solubility values in the acidic environment of the stomach compared with the more neutral environment of the small intestine, thereby leading to a susceptibility to precipitate in the intestine. Precipitation of poorly water soluble drugs can result in erratic absorption and decreased bioavailability. Different authors have shown that due to the lack of an absorptive compartment, an *in vitro* model can over predict the precipitation of a weak base (Heigoldt U,

Sommer F, 2010; O'Dwyer et al., 2018; Ruff A, Fiolka T, 2017; Sironi et al., 2018) Therefore, biphasic dissolution testing has been proposed, which includes dissolution of drug in an aqueous phase and drug partitioning into an organic phase. (O'Dwyer et al., 2018; Shi et al., 2010) The *in vitro* biphasic test use of a lipid layer, such as octanol or decanol, in dissolution testing has been employed to act as a 'quasi-sink' since unionized drug can partition from the aqueous layer into this organic compartment to mimic drug absorption in the intestine. This allows to study simultaneously how a drug is released and taken up.

The dissolution of drug in the aqueous phase determines the amount of drug available for partitioning into the organic phase, which acts as a sink. (Frank et al., 2014a; O'Dwyer et al., 2018; Shi et al., 2010; Xu et al., 2017) Such two phase dissolution tests can offer several advantages due to the presence of a second layer reflecting drug absorption from the intestine. First of all, in the presence of the organic layer, the dissolution profile of the dosage form can be different compared to single phase dissolution systems. A key aspect of the biphasic dissolution is that it mimics the *in vivo* absorptive sink following drug release. The free drug in solution in the aqueous phase provides the driving force for partitioning into the organic layer and the concentration of the drug in the organic phase is a marker of *in vivo* absorption. This is supported by recent studies in which drug concentrations in the organic layer of a biphasic test were found to correlate with *in vivo* drug absorption (Shi et al., 2010; Tsume et al., 2019). These findings are encouraging even though the degree of correlation may depend on the used drug, formulation as well as test conditions.

A particular advantage of biphasic release testing is that direct *in-situ* determination of drug concentration in the organic phase is often less challenging than analytics in the aqueous phase.

This is because of the lack of turbidity compared to the aqueous phase in which particles can originate from non-dissolving excipients or precipitated drug.

Apart from analytics of drug concentrations, it would be of interest to monitor physical changes occurring during drug release from a formulation. Especially measurement of polymer swelling and microrheology upon dissolution testing presents an unmet research need in drug release analysis from solid dispersions. This study introduces diffusing wave spectroscopy (DWS) to ultimately gain a deeper understanding of drug release from polymeric matrices. DWS is an optical technique based on light scattering that investigates the microrheological properties of the fluid based on the intensity correlation function. DWS is a fast and non-contacting method in which the sample is probed by a laser beam over a large frequency range that is partially inaccessible to classical mechanical rheology. It has been just recently introduced into pharmaceutics to characterize lipid-based formulation (Niederquell et al., 2012b; Reufer et al., 2014), but to the best of our knowledge, this is the first time it has been used for amorphous solid dispersions. Ketoconazole (KCZ), a poorly soluble drug and a weak basic drug (basic pKa = 2.9 & 6.5, log P =3.9), was selected as a model compound. Similar to other such bases, KCZ is rather soluble in acidic gastric fluid, but precipitates in intestinal media. (Fricker G., 2012; Warren et al., 2010) In order to reduce or avoid precipitation in the intestinal environment, HPMCAS was employed since it is a polymer that dissolves at pH values higher than 5.5. A number of researchers have highlighted the unique properties of HPMCAS since the polymer is partially ionized above pH 5.5 and therefore becomes a hydrated polyelectrolyte. (Friesen et al., 2008a; Succinate-based et al., 2008) The presence of the charges influences polymer conformation in solution; on one hand it inhibits the formation of big polymer coils, while on the other hand, HPMCAS facilitates the

stabilization of small drug/polymer aggregates that allow the drug to stay in solution. (Friesen et al., 2008a) These unique characteristics make HPMCAS a particularly interesting candidate for ASDs. The aim of this article is to assess the performance of the KCZ ASDs employing a biphasic dissolution test further incorporated a pH shift and to correlate it with microrheology and in particular the swelling of the polymer dispersion by using DWS.

2. Materials and methods

2.1 Materials

Ketoconazole (KCZ) was purchased from BOCSI, Inc. (USA), hydroxypropyl methyl cellulose acetate succinate (HPMCAS) L,M,H grades were obtained from Shin-Etsu Chemical Company (Tokyo, Japan), triethyl citrate (TEC), sodium chloride, sodium acetate trihydrate and sodium dihydrogen phosphate monohydrate was purchased from Sigma Aldrich, (St.Louis MO, USA). The lipid Gelucire 50/13 (Stearoyl macrogol-32-glycerides) was kindly donated by Gattefossé (Luzern, Switzerland). FaSSIF V2 was purchased from Biorelevant.com. (UK) All solutions were prepared using Mill-Q water (18.2 MΩ cm^{-1}).

2.2 Preparation of solid dispersions and physical mixtures

ASDs were prepared by using hot a melt extrusion (HME) method as described in the literature. (Maniruzzaman et al., 2016; Sarode A. , Obara S., 2014) Briefly, KCZ, polymer and plasticizer (Gelucire 50/13 or TEC) were employed in a ratio of 20:80:10 w/w and mixed with the spatula. The different compositions of the extrudates were prepared by the HME process using a Thermo Scientific Haake MiniLab II, which is a conical, co-rotating, twin- screw microcompounder (Thermo Electron, Karlsruhe, Germany). The premix was manually fed into the extruder hopper

and the temperature of the barrel was set to 150°C. The screw speed during the feeding step was 150 rpm. Subsequently, the extrudate strand was allowed to exit from the flat die by opening the bypass valve. The strands were then stored in the desiccator until analysis. Extrudate strands were pelletized using a Thermo Scientific Process 11 (Karlsruhe, Germany) producing pellets of 2mm employed for dissolution.

2.3 Powder X-ray diffraction (PXRD)

PXRD was used to characterize the solid form of the physical mixtures and of freshly prepared ASDs at ambient temperature using a Bruker D2 PHASER (Bruker AXS GmbH, Germany) with a PSD-50 M detector and EVA application software version 6. Samples were prepared by spreading pellets from HME or powder of the physical mixture or collected after filtration at the end of the USP II dissolution test on PMMA specimen holder rings from Bruker. Measurements were performed with a Cu Kα radiation source at 30 kV voltage, 10 mA current and were scanned from 6–40 2θ, with 2θ being the scattering angle at a scanning speed of 0.016θ/min.

2.4 Differential scanning calorimetry (DSC)

A DSC 4000 System, from DSC 3 (Mettler Toledo, Greifensee, Switzerland) was calibrated for temperature and enthalpy using indium. Nitrogen was used as the protective gas (20 mL/min). Samples (approximately 5 mg) were placed in 40 µL aluminium pans with pierced aluminium lids. The midpoint glass transition temperature (T_g), was determined by a single-segment heating ramp of 10 °C/min from 25 °C to a maximum temperature of 200 °C. All DSC measurements were carried out in triplicate.

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149 **2.5 Biphasic dissolution test**

150 The biphasic dissolution experiments were carried out using the inForm (Pion Inc., Billerica, MA)
151 platform, with the experimental setup shown in **Figure 1**. The UV detection wavelengths for
152 ionized (pH 2) KCZ was between 260 and 280 nm, for unionized (pH 6.8) KCZ between 280 and
153 300 nm and in the organic layer the selected range was 285 -315 nm. A linear relationship
154 (R^2>0.99) was established between absorbance and concentration in each of the media tested. KCZ
155 formulations were added into the gelatin capsules and delivered via the sample holder into a
156 cylindrical vessel (diameter 49.9mm, height 74.9mm). Crystalline KCZ and KCZ ASD
157 formulation were delivered at a dose equivalent to 20mg of KCZ . Initially, samples were
158 introduced into 36 mL of 0.01M acetate phosphate at pH 2. After 30 minutes, to replicate the
159 transition into the upper small intestine, 4 mL of 10 x concentrated FaSSIF V2 was added and the
160 pH was adjusted to pH 6.8. A layer of decanol (40mL) was added after reaching pH 6.8. The
161 duration of the intestinal sector was 240 minutes. Concentration of KCZ in both layers was
162 quantified every 2 minutes using two *in situ* multi-wavelength fiber optic UV dip probes. The pH
163 was monitored throughout the experiment using an *in situ* pH probe and controlled to ± 0.1 pH
164 unit using 0.5M HCl or 0.5M NaOH. Temperature was monitored using the temperature probe and
165 controlled to 37ºC using a heating block. The stirring speed of the paddles was set to 100rpm.
166 Stirring was temporarily stopped, while the layer of decanol was added into the vessel**.**

167

168 **2.6 Diffusing wave spectroscopy (DWS)**

169 DWS RheoLab (LS Instruments AG, Fribourg, Switzerland) was used as optical technique for
170 microrheological measurements. (Alexander M, Piska I, 2008; Reufer et al., 2014; Vasbinder A,

2003) The theory of DWS-based microrheology has already been explained in detail in previous works. (Niederquell et al., 2012a; Reufer et al., 2014) The DWS was calibrated prior to each measurement with a suspension of polystyrene particles, PS, (Magsphere Inc., U.S.A) in purified water (10 wt. %). The PS particles have a mean size of 250± 25 nm with a solid content of 0.5 wt. % in dispersion. This suspension was filled in cuvettes with a thickness L of 5 mm prior to measuring for 60 s at 37 °C. The value of the transport mean free path, l^* (microns) was determined experimentally, as reported previously. (Negrini et al., 2017) The transmission count rate was measured several times until a constant value was reached and the cuvette length, L, was considerably larger than the obtained values for l^* ($L \gg l$) ensuring diffusive transport of light. The transport mean free path of the sample l^* is needed for the determination of the correlation intensity function and, thus for the microrheological characterization. Microrheological characterization (l^* and complex viscosity) were performed on the samples withdrawn from the UPS II dissolution test at different time points.

Thus, 0.5 wt. % PS nanoparticles were added to the clear samples to ensure the correct regime (guarantee a L/l* ratio larger than 7). (Reufer et al., 2014) Quartz cuvettes (5 mm) were used and data acquired for 60 s. Each sample was measured 5 times as previous measurements**.** The microrheological characterization of polymer solutions was measured in a broad frequency range by DWS, whereas an average of the complex viscosity, at high frequencies (90 000rad/s) was selected.

2.7 USP II dissolution test

In order to investigate polymer swelling in the aqueous layer with DWS, a dissolution testing using the same proportion between drug amount and the dissolution volume in the biphasic method was

carried out using an Erweka USP II dissolution apparatus (Heusenstamm, Germany). Temperature was set to 37^0C and initial release testing was in 225 ml of 0.01M acetate phosphate buffer at pH 2 for the first 30 minutes, with a stirring speed of 150 rpm. After 30 minutes, samples were withdrawn and transferred in 225 mL of biorelevant fasted state medium FaSSIF V2 at pH 6.8, with the temperature maintained at 37 ^{0}C ± 0.5 and stirring speed of 150 rpm. (Amidon et al., 1995) The biorelevant media were prepared using SIF-powders based on instructions from www.biorelevant.com. Gelatine capsules containing 225 mg of ASDs, equivalent to 45 mg of KCZ were tested. A 5 mL aliquot was withdrawn at appropriate time intervals and replaced with fresh dissolution media. Microrheology of withdrawn samples was determined using DWS as described above.

2.8 Statistical analysis

Analysis of the variance (ANOVA) and regression analysis were calculated using Statgraphics (v16.1.11, Statpoint Technologies, Inc., Warrenton, Virginia). A multi factor ANOVA was used to assess possible effects of polymer grades, plasticizers, and time on drug uptake into the organic phase.

3. Results

3.1 *In vitro* characterization of crystalline KCZ

Figure 2 shows the biphasic dissolution profile of KCZ in aqueous and organic phase, represented as drug concentration (µg/mL) versus time. Drug concentration reaches a peak in the gastric compartment after 0.5h, while at simulated intestinal conditions, the drug concentration decreased

strongly due to precipitation caused by the lower solubility at intestinal pH (pKa 2.9 and 6.5). Precipitation of the drug was assessed using DWS. The parameter monitored was the transport mean free path *l*,* that can be viewed as a measure of sample turbidity. More specifically, *l** is a critical length scale in the case of diffuse light propagation and is described as the distance a photon travels in the sample before its direction of propagation is randomized. Therefore, the lower the value of *l**, the more turbid is a sample. In **Figure 3,** a decrease of *l** indicated turbidity due to precipitated drug upon change to intestinal medium. X-ray diffractograms at 25°C (**Figure 4b**) revealed that after 4.5h of dissolution time, KCZ existed in a crystalline state when compared with the KCZ reference material (**Figure 4a**).

3.2 Bulk characterization of crystalline material and solid dispersions

Prepared ASD, physical mixtures and raw materials were analyzed by PXRD at 25°C to verify the amorphous nature of the dispersions, and the results were compared with those of the corresponding physical mixtures. As shown in **Figure 5,** KCZ based ASDs manufactured with HPMCAS-L, HPMCAS-M and HPMCAS-H grade were amorphous at 20% (w/w) drug loading. In contrast, physical mixtures and raw materials were crystalline, as expected. ASDs were further characterized by DSC (**Figure 6)** to confirm the solid form of the drug in the physical mixture and in the ASDs. All ASDs showed an absence of the KCZ melting peak, while the physical mixture had a melting peak of the drug (T_m).

3.3 Biphasic dissolution experiment of ASDs

Figure 7a depicts dissolution profiles of six different ASDs formulation in the aqueous layer. As mentioned before the first 0.5h of the dissolution test were in a gastric environment and during this

time, no relevant differences were observed between formulations. By contrast, in the intestinal sector, differences between formulations and polymer grades were observed. As a trend, the L polymer grade resulted in comparatively highest amounts of KCZ in solution, followed by the M and H grades even though the given plasticizers appeared to play a role as well. The absolute concentrations in the aqueous phase result in kinetics that is essentially confounded by the overlapping processes of drug release, supersaturation, and optional precipitation. It was therefore important to compare the concentrations profiles also with those obtained from the organic layer, simulating the amount of absorbed drug. **Figure 7b** demonstrates again clear differences between the formulations depending on polymer grade and plasticizer used.

3.4 Microrheological characterization

Analysis of biphasic dissolution test results revealed differences between grades of the HPMCAS polymer. The behavior of the different solid dispersions was further studied in a USP II dissolution vessel using DWS. This microrheological technique allowed even at low polymer concentrations to monitor the mechanical sample dynamics over a large range of time scales (10^{-7} s to 10s) and local displacements. Using DWS, it was possible to correlate the decrease of complex viscosity with an increase of KCZ concentrations over dissolution time in the aqueous layer. The dissolution test was performed at pH 6.8 using the same biorelevant medium FaSSIF V2 and polymer concentration as compared to the biphasic *in vitro* experiment. In **Figure 9** is shown that comparatively higher complex viscosity was noted in gastric conditions (0.5h) with moderate differences between samples. More pronounced differences were seen depending on polymer grade and plasticizer following transfer into the intestinal medium. In **Figure 10,** it can be seen

that values of *l** generally decreased over time, which meant an increase in turbidity. Sample differences were specific for given grades and plasticizers and the residue of the ASDs were studied by PXRD analysis after 4.5 h of dissolution and showed absence of crystalline drug precipitate. (**Figure 8**)

3.5 Statistical analysis

A multi factor analysis of variance (ANOVA) was calculated with respect to the uptake of drug amounts into the organic phase, *Q* (%). The organic phase data were selected for the correlation analysis since lack of particles avoided the complication of turbidity that occurred in the aqueous phase.

The statistical analysis revealed a significant influence of the polymer grades on drug release into the organic phase, as well as another effect of the plasticizer on drug release as indicated by the obtained *p*-values (Table 2). The ANOVA table decomposes the variability of *Q* (%) organic phase into contributions due to various factors. Since type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. Different factor effects as well as interactions were found to be significant with *p*-values lower than 0.05 (i.e. 95% significance level) regarding the drug amount taken up into the organic phase Q (%). (**Table 2)** Inspecting means of drug uptake into the organic layer provided highest values for L grade, then the M grade, and finally the H grade of HPMCAS. The interaction of polymer grade and dissolution time revealed that the above ranking was primarily due to the drug uptake at 4.5h. A comparison of the means for plasticizer effect showed on the average higher uptake for formulations containing Gelucire instead of TEC.

Interesting was a regression of drug amounts in the organic layer versus the aqueous layer, which provided a correlation coefficient of $r = 0.50$ ($p = 0.0002$) if all data were considered and at the longest release time, the correlation was higher $r_{4.5h} = 0.83$ ($p < 0.0001$). These correlations were expected to be less than unity because partitioning into the organic layer is governed by thermodynamic activity rather than drug concentrations or amounts.

In the aqueous phase, the amount released can be compared versus the complex viscosity and resulted in a correlation coefficient of $r = 0.50$ ($p = 0.0001$) and for the longest release time in $r_{4.5h} = 0.67$ ($p = 0.0024$). Interestingly, a negative weak correlation was found for drug amounts released into the aqueous phase versus l^* with $r = -0.31$ ($p = 0.0222$). This correlation coefficient became stronger for a consideration of endpoint data only, $r_{4.5h} = -0.73$ ($p = 0.0005$).

4. Discussion

To cope with the biopharmaceutical challenges of poorly soluble drugs, one of the current research needs is to improve predictive *in vitro* tools, while another important need is to have suitable analytical methods to study mechanistic aspects upon release. This work studied amorphous solid dispersions of KCZ in a biphasic *in vitro* test and introduced DWS to elucidate mechanisms of sustained drug release from ASD.

From the data in **Figure 7**, it is apparent that the ASDs of KCZ displayed a dissolution behavior that was quite distinct from that of crystalline KCZ. (**Figure 2)** Crystalline KCZ completely dissolves in the gastric compartment (>99%) and upon transition into the intestinal medium, precipitation occurred. The dissolved amount in the aqueous phase readily partitioned into the organic layer, providing constant drug concentrations over the dissolution time (plateau is around

200 µg/mL). According to these data, we can infer that drug precipitation in FaSSIF might be considered faster than the partitioning rate into the organic layer. Precipitated fraction of the crystalline KCZ in the intestinal condition was found to be crystalline, in contrast to findings reported by Psachoulias et al. (2011). (**Figure 4**) This study together with findings of Ruff et al. () that precipitation from a non-sink transfer test suggests that the degree of precipitation *in vitro* was higher as compared to the *in vivo* situation. Our results with pure KCZ also showed marked precipitation despite of the given absorptive sink. The rate of transfer or other differences to the *in vivo* intestinal conditions (such as pH, amount of lecithin, bile salts, and rate of absorption) may less precipitation *in vivo* (Psachoulias et al., 2011).

To reduce precipitation of a weak base like the model compound KCZ, the enteric coating polymer HPMCAS was used as matrix in solid dispersions. Release performance was assessed in the biphasic test equipment, while mechanistic aspects were investigated by means of DWS.

Considering the obtained dissolution profiles of ASDs, it is possible to observe that at the end of the simulated gastric phase, the majority of the KCZ (about >75%) from the ASDs remained undissolved. Thus, all ASDs provide significantly lower concentrations of KCZ under gastric conditions when compared with the crystalline KCZ. This was due to the poor solubility of the HPMCAS polymers at gastric conditions at pH 2. The low initial release of KCZ from the ASDs during this gastric phase was hence expected to be mostly caused by KCZ close to the surface of the ASDs since the bulk of the polymer matrix would hardly swell and dissolve in the acidic environment. After transition to intestinal conditions, KCZ released by the ASDs partitioned into the decanol layer, with the L type HMPCAS ASDs resulting in the greatest release of drug whilst the H grade HMPCAS ASDs showing the lowest release of drug, as presented in **Figure 7**. Dissolution performance of each formulation is reflected with the mass of the residue collected at

329 the end of the dissolution test. (**Table 3)** Indeed, H grade reported the highest solid amount
330 undissolved (83.58 % of the ASD with TEC and 69.70% of ASD with Gelucire), while L grade
331 presented the lowest solid amount (24.36% in ASD with TEC and just 3% in the ASD with
332 Gelucire). In addition, ASDs using the Gelucire plasticizer showed a larger release of KCZ
333 compared to the ASDs using TEC as a plasticizer. These higher concentrations of KCZ from the
334 ASDs using Gelucire was a likely consequence of the surfactant properties of Gelucire resulting
335 in greater solubilization of KCZ in the aqueous layer. (Eloy et al., 2012) In accordance with the
336 results in the aqueous layer, the partitioning rate into the organic layer differed greatly among the
337 ASDs. KCZ released from the ASDs partitioned into the decanol layer in an almost linear fashion
338 and reflect the dissolution performance of the ASDs in the aqueous phase. It can be therefore
339 summarized that avoidance of high release in the stomach medium and the observed sustained
340 release in the intestinal medium were advantages of the employed ASD formulations to maximize
341 absorptive drug uptake. This is in line with literature of other solid dispersions that a sustained
342 drug release rate would lead to relatively longer induction times of crystallization, which thereby
343 forms the basis of effective drug absorption. (Frank et al., 2014b, 2012; Sun and Lee, 2013)

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345 Since drug release and hence absorption is governed by rather complex mechanism in the aqueous
346 phase and the drug concentration in the aqueous phase is the driving force for partitioning into the
347 organic layer, it was of interest to obtain complementary data to the kinetics of drug concentrations.
348 We introduced DWS to gain knowledge about microrheological properties of the dispersed ASDs
349 (such as the complex viscosity) and to study l^* over the dissolution time. The mechanism of drug
350 release from ASDs is typically governed by polymer swelling and the dissolution of the carrier in
351 the solvent medium. (Frank et al., 2014b; Sun and Lee, 2013) Such swelling and dissolution is

dependent on ionization state of the polymer as well as on how close its solubility parameter is to that of the surrounding medium. (Jankovic et al., 2018) The selected carrier, HPMCAS is practically not soluble in gastric media, while it starts to dissolve and swell at pH values higher than 5.5 depending on the ratio of succinoyl and acetyl moieties. (**Table 1**) The poor solvent conditions of the acidic medium would theoretically lead to undissolved dispersed polymer particles together with some initial polymer swelling. In addition, at lower pH values the polymer is not charged and the absence of repulsive charge might cause polymer particles aggregation. (Friesen et al., 2008b) Thus, large hydrophobic aggregates of the polymer and drug in the gastric conditions provide higher values of complex viscosity in the medium as evidenced by DWS. (**Figure 9**) The opposite situation is observed in FaSSIF, where the polymer exists as colloidal aggregates. (Frank et al., 2014b) Indeed, in the simulated intestinal media, the colloidal nature of HPMCAS allows the drug to interact with hydrophobic moieties of the carrier proving amorphous drug/nanostructures as reported in literature. (Friesen et al., 2008b; Ricarte et al., 2017a) The negative charge of the succinoyl groups present in the polymer matrix in intestinal media provide stable nanoaggregates due to the repulsive forces and prevent aggregation of the polymer in big nanoaggregates causing a decrease in the complex viscosity in the intestinal conditions compared to the gastric. (Barnes, 2003; Blaabjerg et al., 2018; Friesen et al., 2008a; Ricarte et al., 2017b) Indeed, it is well known that viscosity is influenced by the particle size and smaller colloidal size have a lower resistance to flow, therefore providing lower viscosity values in solution (Barnes, 2003) As reported in literature upon contact with water, Gelucire forms micelles and their presence in solution likely influenced the drop in complex viscosity observed for these formulations. It is known that micelles increase the solubility and wettability of poorly soluble compounds and these might be the reason of higher drug release from ASDs Gelucire formulations. The same trend in

complex viscosity values were also evidenced in comparative experiments with just polymer and plasticizer matrix, indicating that drug had a relatively lower influence on the microrheology compared to the excipients alone (data not shown). Critical for drug release is the particles and colloidal assemblies that emerge from ASDs and a recent study reported on different accessible drug fractions in the aqueous phase of HPMCAS-based solid dispersions. (Aleandri et al., 2018) This study about the accessible drug fraction used fluorescence and suggested that thermodynamic activity in such HPMCAS systems can clearly differ from nominal concentrations. This compares well with the present finding that drug amounts in the aqueous and organic phase shared do not share high correlation especially early in the release process.

The present DWS study further considered the mean free path l^*, which has been used before to study microstructural changes in emulsion system. (Reufer et al., 2014) The mean free path l^* describes the optical characteristics of the sample. In this study it has been employed to describe the appearance of the turbidity during dissolution. In case of pure KCZ decrease of the l^* values was observed during dissolution time probably due to the precipitation upon the transition into intestinal pH. The precipitation behavior of crystalline KCZ in the aqueous phase is also shown during the biphasic dissolution testing with the UV blackout. (**Figure 2**) Considering the dissolution of ASDs, an interesting finding is that in the case of ASDs, there was a drop in l^* at the end of the dissolution test compared to the initial values in gastric medium. **(Figure 10)** In the latter systems, the increase of the turbidity is due to the increase of the polymer drug nanoaggregates occurring during the dissolution time in the aqueous phase. Indeed, it can be observed that lower values of l^* are provided with Gelucire formulations (L and M grade of the polymer) that are also performing better in the biphasic dissolution testing.

Overall the obtained results support the idea that HPMCAS is a good candidate for ASD formulation development of especially weak basic drugs. Indeed, the major advantage of the ASDs formulations is the sustained drug release from the polymer matrix when compared to the performance of the crystalline KCZ. (**Figure2**)

ASDs formulations with HPMCAS have been prepared employing solvent methods, but some researchers have also studied the utility of HPMCAS for HME. (Sarode et al., 2013; Tanno et al., 2004) Optimization of the process was meaningful to avoid degradation of drug or polymer in line with Sarode et al. (2014) who have investigated the stability of HPMCAS for HME. The present study accordingly extruded at a temperature (i.e. 150°C) that was deemed as uncritical from a degradation viewpoint, which was enabled by adding suitable plasticizers (i.e. Gelucire 50/13 or TEC). Process optimization might be critical for the miscibility of the system and therefore for the stability of the formulations. All ASDs formulations appear miscible, presenting just one single T_g. (**Figure 6**) Miscibility is an important factor for physical drug stability and all ASDs presented the drug in the amorphous form. **(Figure 5)** Even though ASDs can be technically produced by employing both TEC or Gelucire as plasticizer, the release results favored ASDs with the PEGylated lipid Gelucire. Increased drug release from these formulations might be due to the multiple interactions between the drug and polymer/lipid matrix compared to the polymer/TEC carrier. (**Figure 7)** (Konno H, 2006; Maniruzzaman et al., 2016; Potluri et al., 2011)

In summary, this study highlighted the importance of the biphasic test dissolution with a pH shift to correlate *in vitro* drug release and precipitation of poorly soluble weak basic drugs with the gastrointestinal absorption in humans. DWS was introduced as an analytical method and was found

to be particularly suitable to correlate microrheological properties of the ASDs into the dissolution media.

Conclusions

Solid drug dispersions form complex and heterogeneous systems upon aqueous dispersions. Course as well as colloidal drug/polymer particles evolve and impact on drug release that in turn drives absorptive flux. Traditional release testing often neglect important aspects like an absorptive sink and they offer only limited physical characterization. More recent approaches attempted to better understand emerging particles that are formed in aqueous environment. The current work used a biphasic dissolution test with a pH shift method and introduced DWS as novel analytical tool to gain a deeper understanding of the dissolution process from ASDs. Information about the polymer matrix was obtained regarding the polymer swelling in aqueous media and differences between the grades and plasticizers were highlighted. The microrheological correlations with drug release provided valuable aspects that were in previous research neglected due to lack of data. More results of further solid dispersions should clarify how broadly the identified correlations hold but already now it can be concluded that DWS is a promising tool for *in vitro* testing of ASDs and potentially also further supersaturating formulations.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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603

Tables

Table 1. Characteristics of HPMCAS L, M and H grades

Grade	MeO	HPO	Acetyl (%)	Succinoyl (%)	Dissolving pH
AS-L	20.0–24.0	5.0–9.0	8	15	>=5.5
AS-M	21.0–25.0	5.0–9.0	9	11	>=6.0
AS-H	22.0–26.0	6.0–10.0	12	7	>=6.5

Table 2. Analysis of variance for Q (%) organic phase - Type III sums of squares

Source	Sum of squares	Df	Mean square	F-ratio	*p*-value
A:HPMCAS	1605.5	2	802.8	108.9	0.0000
B:Plasticizer	190.3	1	190.3	25.8	0.0000
C:time (h)	15696.4	2	7848.2	1065.2	0.0000
INTERACTIONS					
AB	17.9	2	8.9	1.2	0.3074
AC	3212.0	4	803.0	109.0	0.0000
BC	319.7	2	159.9	21.7	0.0000
RESIDUAL	294.7	40	7.4		
TOTAL (CORRECTED)	21336.6	53			

Table 3. Outline of the calculated percentage (w/w) KCZ in each layer at the end of the biphasic dissolution experiment; formulations with Gelucire as plasticizer are indicated with Gel, while the others with triethyl citrate are indicated with TEC

	Percentage of 20mg KCZ Dose (%)		
Formulation	Organic layer	Aqueous layer	Solid
L Gel	66.24	30.88	2.88
M Gel	51.72	28.41	19.87
H Gel	22.34	7.96	69.70
L TEC	53.38	22.26	24.36
M TEC	42.44	16.32*	41.24
H TEC	11.24	5.18*	83.58
Crystalline	45.76	2.99**	51.25

**Taken from last time-point prior to UV blackout due to the turbidity occurring in the aqueous phase. **Estimated using equilibrium solubility data.*

Figure captions

Figure 1: Schematic representation of the biphasic dissolution setup

Figure 2. Dissolution profile of KCZ in the biphasic dissolution test: aqueous profile (filled circles) and organic profile (opened circles). Broad error bars and the connecting dotted line are an indication of UV blackout occurring in the aqueous phase due to rapid KCZ precipitation.

Figure 3. Microrheological characterization of the aqueous dissolution profile of pure KCZ with DWS in the USP II apparatus

Figure 4. KCZ raw material (a), ketoconazole precipitate after 4.5h of dissolution test at 37 degrees (b)

Figure 5. X-ray powder diffraction patterns of Gelucire (a) KCZ (b), physical mixtures of HPMCAS H, Gelucire, and KCZ (c) Physical mixtures of HPMCAS M, Gelucire, and KCZ (d), physical mixtures of HPMCAS L, Gelucire, and KCZ (e), physical mixtures of HPMCAS H, TEC, and KCZ (f), physical mixtures of HPMCAS M, TEC, and KCZ (g), physical mixtures of HPMCAS L, TEC, and KCZ (h), ASD HPMCAS H, Gelucire, and KCZ (i), ASD HPMCAS M, Gelucire, and KCZ (j), ASD HPMCAS L, Gelucire, and KCZ (k), ASD HPMCAS H, TEC, and KCZ (l), ASD HPMCAS M, TEC, and KCZ (m), ASD HPMCAS L, TEC, and KCZ (n).

655

656

657 **Figure 6.** DSC thermograms of ASD HPMCAS L, TEC and KCZ (a), ASD HPMCAS M, TEC,
658 and KCZ (b), ASD HPMCAS H TEC and KCZ (c), ASD HPMCAS L, Gelucire, and KCZ (d),
659 ASD HPMCAS M, Gelucire, and KCZ (e), ASD HPMCAS H, Gelucire, and KCZ (f), physical
660 mixtures of HPMCAS L, TEC, and KCZ (g), physical mixtures of HPMCAS M, TEC, and KCZ
661 (h), physical mixtures of HPMCAS H, TEC, and KCZ (i), physical mixtures of HPMCAS L,
662 Gelucire, and KCZ (j), physical mixtures of HPMCAS M, Gelucire, and KCZ (k), physical mixture
663 HPMCAS H, Gelucire, and KCZ(m), HPMCAS L (n), HPMCAS M (o), HPMCAS H (p), TEC(r),
664 Gelucire (q), KCZ alone (t)

665

666 **Figure 7.** Biphasic dissolution test in aqueous (a) and in organic layer (b). Filled symbols indicate
667 ASD Gelucire formulation with L grade (triangle), M grade (square), H grade (circles) while
668 opened symbols represent ASD TEC formulation with L grade (triangle), M grade (square),H
669 grade (circles). Some aqueous layer dissolution profiles appear not complete because of the strong
670 turbidity blocking UV light reaching the detector. KCZ release was studied in triplicate for each
671 formulation and means are given +/- SD.

672

673 **Figure 8.** Representative X-Ray diffraction patterns of KCZ precipitates. KCZ reference material
674 a), crystalline pattern collected from the intestinal sector (*in vitro*) that refers to pure KCZ after

675 4.5h of dissolution at 37 ^{0}C b), amorphous patterns that refer to collected solid from ASDs in the
676 intestinal sector (4.5 h, 37°C) that indicate absent crystallization c),d),e),e),f),g).

677

678 **Figure 9.** Representative microrheological characterization employing complex viscosity, η^*
679 versus dissolution time (h) for different grades of HPMCAS. Filled symbols indicate ASD
680 Gelucire formulation L grade (triangle), M grade (square), H grade (circles) while opened symbols
681 represent ASD with TEC L grade (triangle), M grade (square),H grade (circles).

682

683 **Figure 10.** Representative microrheological characterization employing the transport mean path,
684 l^* versus dissolution time (h) for different grades of HPMCAS. Filled symbols indicate ASD
685 Gelucire formulation L grade (triangle), M grade (square), H grade (circles) while opened symbols
686 represent ASD with TEC L grade (triangle), M grade (square), H grade (circles).

687