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Investigation of the formation of drug-drug cocrystals and coamorphous systems of the antidiabetic drug gliclazide

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Abstract

The antidiabetic drug gliclazide (GLZ) has a slow absorption rate and a low bioavailability due to its poor solubility. GLZ is often prescribed along with an antihypertensive, as many diabetic patients have coexistent hypertension. Cocrytallization and coamorphization are attractive strategies to enhance dissolution rates and to reduce the number of medications a patient has to take. In this work the formation of cocrystals and coamorphous systems of GLZ with various antihypertensive drugs was studied, namely chlorothiazide (CTZ), hydrochlorothiazide (HTZ), indapamide (IND), triamterene (TRI) and nifedipine (NIF) as well as benzamidine (BZA) as a model for the amidine pharmacophore. TRI, IND and HTZ were found to form coamorphous systems with GLZ that are stable for at least six months at 22 ± 2 °C and 56 % relative humidity. Coamorphous GLZ-TRI is also stable in dissolution medium. Coamorphization of GLZ-TRI with 15 % sodium taurocholate gave a viable coamorphous formulation with an enhanced dissolution rate. Comilling of GLZ with BZA and cocrytallization from solution gave the amorphous and crystalline salt, respectively and the X-ray structure is reported. During attempts to obtain X-ray suitable cocrystals crystals of
Na⁺\text{GLZ} \text{ and IND-0.5H}_2\text{O} \text{ were obtained. Redetermination of the published structure of IND-0.5H}_2\text{O revealed a unit cell with the length of the } a \text{ axis doubled, a different space group and no disorder. Liquid-assisted grinding of a 1:1 mixture of GLZ and IND indicated the transformation of IND to a new solid-state form, while GLZ remained unaltered. Milling- and heating-induced solid-state transformations of IND are discussed.}

\textit{Keywords:} Amorphous; Antidiabetic; Dissolution studies

1. Introduction

Gliclazide (GLZ, Scheme 1) is an oral hypoglycemic drug. It is used for the long-term treatment of non-insulin dependent diabetes mellitus and is listed on the WHO model list of essential medicines (20\textsuperscript{th} WHO Model List of Essential Medicines; WHO, March 2017). As a BCS (biopharmaceutics classification system) class II drug (low solubility, high permeability) GLZ has a slow absorption rate and low bioavailability due to its poor solubility. Various approaches to improve the dissolution behaviour of GLZ have been pursued including micronization (Varshosaz et al., 2008), encapsulation into nanoparticles (Devarajan and Sonavane, 2007; Naik et al., 2013), dispersion into polymers (Hong et al., 1998; Biswal et al., 2008, 2009a,b; Jondhale et al., 2012; Lu et al., 2017), salt formation (El-Sabawi and Hamdan, 2014; Putra et al., 2016a,b; Samie et al., 2017) and complexation with cyclodextrins (Winters et al., 1997; Özkan et al., 2000; Aggarwal et al., 2002). More recently, a limited number of studies on GLZ cocrystals were also reported (Chadha et al, 2016, 2017; Samie et al., 2017; Bruni et al., 2018). Chadha and coworkers used the GRAS (Generally Recognized as Safe) coformers succinic acid, malic acid, sebatic acid and α-hydroxyacetic acid to obtain multicomponent crystals of GLZ with enhanced dissolution properties (Chadha et al., 2016, 2017). The cocrystallization of GLZ with catechol and resorcinol was studied by Desiraju and coworkers (Samie et al., 2017). Bruni et al. reported a multicomponent crystal of GLZ and the excipient tromethamine (Bruni et al., 2018). Uekusa and coworkers cocrystallized GLZ with the antidiabetic drug metformin and obtained a salt with a significantly improved solubility and dissolution rate (Putra et al., 2016a).
Coamorphization has recently emerged as a new and promising formulation strategy for poorly soluble drugs and takes advantage of the inherent higher apparent solubility of an amorphous phase compared to its crystalline counterpart (Grohganz et al., 2014; Laitinen et al., 2017; Newman et al., 2018). The term ‘coamorphous system’ is usually used in the literature for a single-phase amorphous, stoichiometric mixture of two active pharmaceutical ingredients (APIs) or of an API and an inactive, biologically safe small-molecule coformer. The coformer stabilizes the (thermodynamically unstable) amorphous phase towards recrystallization by physically separating the API molecules and/or preventing the reorientation of the API molecules through the formation of API-coformer intermolecular interactions. Coamorphization becomes an even more powerful approach, when the inactive coformer is replaced with a complementary drug (Laitinen et al., 2013; Chavan et al., 2016). Besides overcoming dissolution and bioavailability issues, a pharmaceutically appropriate coamorphous system reduces the number of medications a patient has to take, thus improving patient compliance and quality of life. While a number of drug-drug coamorphous systems have been developed (Chavan et al., 2016), the focus has been on preparation, characterization and stabilization of the amorphous phase. Significant progress has been made on the selection of suitable coformers in this regard. However, it is not an uncommon problem that the inherent higher apparent solubility of an amorphous material is offset by poor dispersibility or wettability (Gniado et al., 2016). Furthermore, so far little attention has been paid to the design of drug-drug coamorphous systems with a pharmacologically relevant dose ratio (Shi et al., 2018). When drug complementarity is taken into account – both in terms of indication and relative dose – the development of viable coamorphous formulations has remained a challenging task.

The aim of the present study was to investigate possible coformulations of gliclazide with an antihypertensive drug. Many diabetic patients have coexistent hypertension which increases the risk of macro- and microvascular complications such as coronary heart disease, retinopathy and nephropathy. The antihypertensives selected in this work were chlorothiazide (CTZ), hydrochlorothiazide (HTZ), indapamide (IND), triamterene (TRI) and nifedipine (NIF) (Scheme 1) that - like GLZ - are BCS class II. Benzamidine (BZA) was included as a model for the important amidine pharmacophore that is present in a wide range of pharmaceuticals including cardiovascular and antidiabetic drugs.
Scheme 1. Chemical structures of GLZ, CTZ, HTZ, IND, TRI, NIF and BZA.

2. Materials and methods

2.1 Materials

Gliclazide, nifepidine, triamterene, indapamide, chlorothiazide and hydrochlorothiazide were purchased from Tokyo Chemical Industry (TCI, Europe). Benzamidine, sodium metasilicate and sodium dodecyl sulfate were purchased from Sigma Aldrich. Sodium taurocholate was supplied by Biosynth. The solvents acetonitrile, diethyl ether, dichloromethane and methanol (Merck Millipore), chloroform (Honeywell), dimethylsulfoxide, tetrahydrofuran (Sigma Aldrich), ethanol, dimethylformamide and acetone (Fisher Scientific) were analytical grade and used as received.

2.2 Methods

2.2.1 Ball milling

Room temperature milling experiments were performed using an oscillatory ball mill (Mixer Mill MM400, Retsch GmbH & Co., Germany) and a 25 mL stainless steel milling jar containing one 15 mm diameter stainless steel ball. The antidiabetic GLZ and the respective antihypertensive were physically mixed in a 1:1, 1:5 or 5:1 molar ratio (0.25 g sample in total). The samples were milled at 25 Hz for up to 180 min. with a cool down period of 15
min. after every 30 min. of milling. For liquid-assisted grinding 100 µL of acetonitrile or methanol was added to the milling jar before milling for 30 min. with a 10 min. break after 15 min. Cryomilling was carried out by immersing the jars in liquid nitrogen for 5 min. prior to milling. After every 7.5 min. of milling the sample was cooled again in liquid nitrogen for 2.5 min. Details of the sample preparations can be found in Table S1. The milled powder samples were analyzed immediately by X-ray powder diffraction. To monitor the recrystallization of the amorphous materials, the samples were stored in a desiccator at ambient temperature (22 ± 2 °C) under 56 % and 98 % relative humidity (RH) which was achieved using solutions of K2SO4 of different concentrations (Lu and Chen, 2007). The stored samples were analyzed after 1, 3, 7, 15, 30, 60 and 180 days by X-ray powder diffraction.

2.2.2 Solution crystallization

Equimolar quantities of GLZ (50 mg, 0.17 mmol), NaOH (6.16 mg, 0.17 mmol) and BZA (18 mg, 0.17 mmol) were weighed in separate vials and dissolved in a minimum amount of methanol. The clear solutions were mixed and left to slowly evaporate at room temperature. X-ray suitable single crystals of (BZA+) (GLZ-) were obtained within a week.

Cocrystallization experiments of GLZ with IND, HTZ, CTZ, TRI and NIF were carried out at room temperature and 4 °C using different molar ratios (1:1, 1:2, 1:3, 1:4, 2:1 and 4:1) and different solvents (Table S2). Both components crystallized separately from all common organic solvents. In an attempt to obtain cocrystals by solvent diffusion, powdered mixtures were dissolved in ethanol. The vial containing the ethanol solution was transferred to a larger vial containing diethyl ether. Again, the two APIs crystallized separately.

X-ray suitable crystals of IND·0.5H2O were isolated after mixing GLZ and IND in a 1:2 ratio in methanol. Na+GLZ- crystallized from silica gel during an attempt to obtain a GLZ-HTZ cocrystal. 1.22 g of Na2SiO3 was dissolved in 5 mL distilled water and stirred for 24 h at room temperature. The metasilicate solution was filtered by pressure filtration. GLZ (16 mg, 0.05 mmol) was dissolved in 0.5 M acetic acid and titrated with the sodium metasilicate solution under continuous stirring until pH 5.5 was reached. The solution was transferred immediately to the crystallization vial which was closed and stored overnight. Then a solution of HTZ (0.05 mmol) in methanol was added. Single crystals of Na+GLZ- were obtained within a week.

2.2.3 Dissolution studies
All samples used in the dissolution studies were gently ground before use to avoid any bias from large particles. The powder sample (100 – 178 mg in total, Table S3) was placed in 250 mL 0.1 M phosphate buffer (pH 6.8, 37 °C) and stirred at 300 rpm with an 11-mm magnetic stirring bar. Aliquots of 2.5 mL were withdrawn at predetermined time points (1, 2, 5, 10, 15, 25, 30, 45, 60, 90, 120, and 180 min.) and immediately replaced with 2.5 mL of dissolution medium. 250 µL of the aliquot was diluted by adding 4.75 mL dissolution medium. For dissolution testing in the presence of a surfactant 150 µL of the aliquot was diluted with 4.85 µL dissolution medium. The solutions were analyzed on the same day using UV/Vis spectroscopy. All dissolution experiments were performed in triplicate. The amount of dissolved GLZ, HTZ, and TRI was determined with a Varian Cary 50 Scan Spectrophotometer (Santa Clara, CA, USA). To exclude any interference due to overlapping absorbances, reference spectra were recorded for the buffer solution and the buffer solution containing either of the components. GLZ concentrations in dissolution studies of GLZ and (BZA⁺)(GLZ⁻) were measured at 226 nm and 238 nm, respectively. In the case of GLZ-TRI and GLZ-HTZ overlapping absorbances were treated by measurements at two suitable wavelengths (Table S3) and simultaneous analysis (Sawyer et al., 1984). Standard solutions were prepared with phosphate buffer (0.1 M, pH 6.8). The resulting calibration curves were linear in the relevant concentration range.

2.2.4 Differential scanning calorimetry

A STA625 thermal analyser from Rheometric Scientific (Piscataway, New Jersey) was used to perform thermal analysis. The heating rate was 10 °C min⁻¹ and the runs were performed between 25 and 400 °C. Open aluminium crucibles were used, nitrogen was purged in the ambient mode and an indium standard was used for calibration.

2.2.5 IR spectroscopy

FT-IR spectra were recorded on a Perkin Elmer Spectrum 400 fitted with an ATR reflectance attachment. Spectra were collected in the 650 – 4000 cm⁻¹ range with a resolution of 4 cm⁻¹ and four integrated scans on a diamond/ZnSe window. Calculated spectra of equimolar physical mixtures of amorphous GLZ and amorphous IND, HTZ, CTZ, NIF, and TRI were generated by averaging the separately collected, standard normal variate (SNV) transformed spectra of both amorphous components.

2.2.6 X-ray powder diffraction
X-ray powder patterns of samples obtained by mechanical grinding and crystallization from solution were recorded on an Inel Equinox 3000 powder diffractometer between 5 and 90° (2θ) using Cu Kα radiation (λ = 1.54178 Å, 35 kV, 25 mA). Theoretical powder patterns of the salts and of IND-0.5H₂O analyzed by single crystal X-ray analysis were calculated using the Oscair software package (McArdle, 2017).

2.2.7 Single crystal X-ray diffraction

An Oxford Diffraction Xcalibur system was used to collect X-ray diffraction data at room temperature (Table 1). The crystal structures were solved using ShelxT and refined using Shelxl 2016/6 within the Oscair package (Sheldrick, 2015a,b; McArdle, 2017). CIF files can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, Cambridge, UK with the REF codes CCDC 1876658, CCDC 1876659 and CCDC 1876660.

3. Results and discussion

The antihypertensives were selected from different compound classes with different functional groups. CTZ and HTZ represent thiazide diuretics whose common feature is a benzothiazide ring system with a sulfonamide substituent. IND contains a primary sulfonamide group, an amide group and an indole ring. TRI and NIF are pteridine and 1,4-dihydropyridine derivatives, respectively. BZA as a model for the amidine pharmacophore represents a strongly basic coformer that may lead to salt formation. As a starting point a 1:1 molar ratio of GLZ and the coformer was applied to gain a better insight into potential intermolecular drug-drug interactions. When coamorphous systems were successfully obtained, pharmaceutically relevant ratios based on the average prescribed doses were studied (GLZ:HTZ 5:1 and GLZ:CTZ 1:5). In the case of IND this would require a 70:1 ratio. Although this is not compatible with the general definition of a coamorphous system we included 1:1 GLZ/IND as a model system because of the known stability of the amorphous phase of IND (Wojnarowska et al., 2013).

Coamorphous systems and cocrystals can be prepared by various methods (Chavan et al., 2016). In this work, ball-milling and liquid-assisted grinding, i.e. mechanochemical syntheses, were used as solvent-free and low-solvent, ‘green’ methods. GLZ and the
respective antihypertensive drug were physically mixed and ball-milled for up to 180 min. in the absence or presence of a drop of solvent. The milled samples were analyzed by X-ray powder diffraction (XRPD) directly after milling and after storage for up to six months. In addition solution cocrystallization experiments were carried out in an attempt to obtain X-ray suitable single crystals. GLZ, CTZ, HTZ, IND, TRI, NIF and BZA were also milled individually for comparison.

3.1 Ball-milling of GLZ, CTZ, HTZ, IND, TRI, NIF and BZA

After dry milling, the XRPD patterns of HTZ and IND showed amorphous halos (Fig. S1). The IND sample remained amorphous for at least ten months during storage at 56 % RH in line with a recent study by Wojnarowska et al. (Wojnarowska et al., 2013), while small crystalline peaks of HTZ appeared after three months. GLZ, CTZ, TRI, NIF and BZA did not amorphize on ball-milling and the XRPD patterns were unchanged. Amorphous CTZ, NIF and TRI, however, were obtained when the milling was performed at low temperature, but recrystallized within 1 d at 56 % RH (Fig. S2). By contrast GLZ and BZA did not convert to the amorphous phase. Amorphous GLZ prepared by melt-quenching was stable for 1 d, but was almost completely recrystallized after a week.

3.2 Ball-milling of binary mixtures

The XRPD patterns of the 1:1 mixtures GLZ-CTZ, GLZ-HTZ, GLZ-IND, GLZ-TRI, GLZ-NIF and GLZ-BZA all showed an amorphous halo after ball-milling for 120 min. (Figs. 1, 2, S3 and S4). GLZ-NIF crystallized to a physical mixture of the two components after 3 d at ambient temperature and 56 % RH (Fig. S3). By contrast, in the case of GLZ-IND, GLZ-TRI and GLZ-HTZ no Bragg peaks were observed after storage for six months under the same conditions (Fig. 1). Even at 98 % RH, GLZ-IND remained X-ray amorphous for at least four months, while sharp GLZ peaks appeared in the XRPD pattern of GLZ-HTZ after one month under these conditions (Fig. S5). The XRPD pattern of GLZ-TRI showed small peaks of crystalline GLZ after storage for four months at 98 % RH (Fig. S5d). Crystallization of GLZ-CTZ stored at 56 % RH started after one month (Fig. 2). After storage at 98 % RH for one month, XRPD analysis of GLZ-BZA (Fig. S4) showed a new pattern that could be assigned to the salt (BZA⁺)(GLZ⁻) (vide infra).
**Fig. 1.** XRPD patterns of milled binary mixtures (1:1 molar ratio): (a) GLZ-IND, (b) GLZ-TRI, and (c) GLZ-HTZ. Left: directly after ball-milling for 120 min. Right: after storage for six months (22 ± 2 °C, 56 % RH).

**Fig. 2.** XRPD pattern of milled GLZ-CTZ (1:1 molar ratio): (a) directly after ball-milling for 120 min. (b) after storage for one month (22 ± 2 °C, 56 % RH).

The co-milled, amorphised samples were also analysed by IR spectroscopy in order to examine drug-drug intermolecular interactions (Fig. S6). Crystalline GLZ gives a characteristic $\nu$(C=O) peak at 1707 cm$^{-1}$ and a split peak at 2950/2935 cm$^{-1}$. In the IR spectrum of amorphous GLZ the latter merges to a single peak at 2950 cm$^{-1}$, while the C=O
band decreases in intensity. The $\nu_s$(SO$_2$) and $\nu_{as}$(SO$_2$) bands of crystalline GLZ at 1162 and 1346 cm$^{-1}$ broaden and shift to 1152 and 1335 cm$^{-1}$, respectively. The IR spectrum of amorphous IND shows characteristic peaks at 3260, 3060, 1660 and 1243 cm$^{-1}$. Compared to crystalline IND the $\nu_{as}$(SO$_2$) band at 1338 cm$^{-1}$ is broadened and has a lower intensity due to the loss of long-range order. It has been noted in the literature that the peak at 1660 cm$^{-1}$ in the IR spectrum of amorphous IND is indicative of a proton transfer from the nitrogen to the carbonyl oxygen and an amide-imidine tautomeric equilibrium on amorphisation (Wojnarowska et al., 2013). The IR spectrum of co-milled GLZ-IND matches the calculated spectrum of a physical mixture of amorphous GLZ and IND (Fig. S6). Likewise, the IR spectra of co-milled GLZ-HTZ, GLZ-CTZ and GLZ-NIF are indistinguishable from the theoretical spectra of the physical mixtures of the respective amorphous components. Thus, the IR data of these coamorphous systems give no evidence for specific H bonding interactions between the two drug molecules. The formation of stable single-phase coamorphous systems in which molecular level mixing is sufficient to stabilize the amorphous state towards recrystallization has been described in the literature (Löbmann et al., 2012; Gniado et al., 2017). By contrast, there are clear differences between the IR spectrum of coamorphized GLZ-TRI and that calculated for physically mixed amorphous GLZ and TRI (Fig. S6) suggesting the formation of new H bonds. A new peak appears in the $\nu$(N-H) region at 3290 cm$^{-1}$ and the NH$_2$ bending vibrations at 1565 and 1530 cm$^{-1}$ are shifted to 1570 and 1536 cm$^{-1}$. The $\nu$(C=O) band of GLZ of the co-milled sample is observed at 1701 cm$^{-1}$ compared to 1707 cm$^{-1}$ for the physical mixture.

Next the amorphization of mixtures containing pharmaceutically appropriate ratios was examined. GLZ and HTZ mixed in a 5:1 molar ratio turned fully amorphous on milling (Fig. S7). However, the milled sample proved less stable towards recrystallization than the 1:1 coamorphous system. Peaks of GLZ appeared in the XRPD pattern after storage for one week at 56 % RH. Milling at room temperature and cryomilling of 1:5 GLZ-CTZ mixtures led to partial amorphization only. Broad peaks of CTZ and an underlying amorphous halo were seen in the XRPD pattern (Fig. S8). The pharmaceutically relevant ratio of GLZ and TRI is approximately 1:1 so that no other GLY-TRI mixtures were examined, while studies of relevant 70:1 GLZ-IND mixtures were not considered meaningful.

As it is well known that milling in the presence of small amounts of solvent (liquid-assisted or solvent-drop grinding) can catalyse cocrystallization, GLZ-HTZ, GLZ-CTZ, GLZ-IND,
GLZ-TRI and GLZ-NIF (1:1) were also milled with a drop of methanol and acetonitrile. No cocrystal formation was observed for GLZ-HTZ, GLZ-CTZ, GLZ-TRI and GLZ-NIF in the presence of methanol or acetonitrile. The XRPD patterns of the milled samples were those of physical mixtures of the starting components. Co-milling of GLZ with IND in the presence of methanol or acetonitrile had no effect on the GLZ peaks in the XRPD pattern indicating that GLZ remains unaltered (Fig. S9). By contrast, IND apparently underwent a solvent-mediated transformation to a new solid-state form as discussed further below. Milling of BZA and GLZ in the presence of methanol gave crystalline (BZA\(^+\))(GLZ\(^-\)) (Fig. S4, *vide infra*).

### 3.3 Solution crystallization experiments

In an attempt to obtain cocrystals from solution, solution crystallization experiments were carried out (Table S2). From a methanolic solution containing equimolar amounts of GLZ, NaOH and BZA crystals grew within a week. Single crystal X-ray analysis indicated that proton transfer had taken place form the sulfonamide nitrogen to the amidine group leading to the formation of the benzamidinium salt (BZA\(^+\))(GLZ\(^-\)) (Fig. 3). There is extensive H bonding between the amidinium group and the sulfonamide and urea oxygens (Table S4). N5-H2 forms a bifurcated H bond to the sulfonyl O2 and carbonyl O3 oxygens giving rise to an \(R_2^1(6)\) motif (N5···O2 = 3.260(3) Å, N5···O3 = 2.919(2) Å). O2 on the other hand acts as a bifurcated H bond acceptor in an \(R_2^1(6)\) motif (N4···O2 = 2.808(3) Å; N5···O2 = 3.260(3) Å). The ureyl O3 oxygen accepts an additional H bond from another BZA\(^+\) cation (N5···O3 = 2.828(2) Å). H bonding between sulfonyl O1 and amidinium N4 generates \(R_4^1(12)\) rings (N4···O1 = 2.926(3) Å). Furthermore, two ureyl oxygens and two amidinium NH\(_2\) groups form eight-membered \(R_4^1(8)\) motifs. Overall, the benzamidinium cations and GLZ anions are packed in chains of fused \(R_2^1(6)\), \(R_2^2(6)\), \(R_4^1(12)\) and \(R_4^1(8)\) rings. Consistent with salt formation, there are clear differences in the IR spectrum of (BZA\(^+\))(GLZ\(^-\)) and those of the starting compounds (Fig. S10). The ν(C=O) and ν\(_{as}(\text{SO}_2)\) bands that appear at 1707 and 1346 cm\(^{-1}\) in the spectrum of GLZ, are observed at 1684 and 1351 cm\(^{-1}\). Pronounced changes also occur in the amine region of BZA. The band at 3170 cm\(^{-1}\) decreases in intensity and the broad band at 3331 cm\(^{-1}\) shifts to 3295 cm\(^{-1}\).
Unfortunately, GLZ and IND, GLZ and HTZ, GLZ and CTZ, GLZ and NIF as well as GLZ and TRI crystallized separately from all common organic solvents. Applying the gel-diffusion method was also unsuccessful. Gel diffusion under basic (NaOH) conditions gave X-ray suitable crystals of the sodium salt of GLZ whose structure has not been previously reported in the literature and is shown in Fig. 4. In Na⁺GLZ the Na⁺ cations are surrounded by three GLZ anions that bind in a chelating mode via three different sites; the sulfonyl O2 / ureyl O3 oxygens (six-membered chelate ring), deprotonated sulfonamide N1 / sulfonyl O1 (four-membered chelate ring) and ring nitrogen N3 / ureyl O3 (five-membered chelate ring). The anions are further linked through N2H···O2 H bonding (Table S5).

Fig. 3. Crystal structure of (BZA⁺)(GLZ⁻).
Our cocrystallization experiments with IND gave single crystals whose XRPD pattern matched that of commercial IND. According to the European Pharmacopeia IND may contain up to 3 wt % of water. The first structure solution of commercial IND was obtained from powder data and was published in 2006 (Smrkolj and Menden, 2006). The structure was refined to IND·0.35H₂O and appeared to confirm the hypothesis that IND exists as a non-stoichiometric hydrate with the water molecules being only weakly bound into the crystal structure. Ten years later a single crystal structure analysis of IND was reported (Bojarska et al., 2016). The unit cell was determined as \(a = 15.0586(9) \ \text{Å}, b = 9.6218(6) \ \text{Å}, c = 23.5080(14) \ \text{Å}, \beta = 92.5980(16)^\circ\) and the structure was solved in space group I2/a as IND·0.5H₂O with the sulfonamide group being disordered over two positions. We redetermined the structure and found a unit cell with the length of the \(a\) axis doubled \((a = 30.0598(11) \ \text{Å}, b = 9.6685(3) \ \text{Å}, c = 23.5727(10) \ \text{Å}, \beta = 92.325(4)^\circ)\), space group P2_1/c and four IND molecules and two water molecules of crystallization in the asymmetric unit (Fig. S11, Table S6). The theoretical XRPD pattern calculated from the single crystal data matched the experimental pattern of the commercial sample (Fig. S12). Smrkolj and Menden observed a broad endotherm below 100 °C in the differential scanning calorimetry (DSC) thermogram of IND·0.5H₂O indicating a continuous loss of water without any indication of a phase transformation during dehydration. XRPD supported the interpretation that the crystal structure did not collapse. Heating to 160 °C where dehydration was anticipated to be
complete, followed by cooling and exposure to air showed that the desolvation is reversible (Smrkolj and Menden, 2006). While we could reproduce the DSC experiment, we observed a new XRPD pattern after heating for 24 h at 160 °C (Figs. S13, S14). This pattern is identical to the one reported in the literature for the solid-state form denoted as IND form I that was obtained when IND was precipitated from glacial acetic acid and is believed to be an anhydrous form (Ghugare et al., 2010). The DSC of our heated sample showed the melting endotherm at 197.5 °C (Fig. S15), slightly higher than the melting endotherm reported for IND form I (189 °C) (Gughare et al., 2010). In our hands IND form I remained stable for four months at 98 % RH.

3.4 Dissolution studies

Dissolution studies were carried out for the two coamorphous systems that were pharmaceutically meaningful and stable at 56 % RH; GLZ-HTZ (1:1) and GLZ-TRI (1:1). The dissolution profiles of amorphous GLZ, HTZ, and TRI were also measured for comparison. Fig. 5a shows the dissolution profiles of coamorphous GLZ-HTZ, crystalline HTZ and crystalline GLZ at pH 6.8. The dissolution profiles of amorphous GLZ and HTZ can be found in the Supplementary Data (Fig. S16). In line with the literature (Jhondale et al., 2012), no improvement of the dissolution properties of amorphous GLZ compared to the crystalline drug was observed. In fact, amorphous GLZ dissolved at a slower rate, probably due to poor wettability and dispersibility, as pronounced clumping occurred during the dissolution testing. Likewise, amorphization of HTZ does not give a dissolution advantage over its crystalline form. The XRPD pattern of the undissolved residue indicated that the amorphous phase had recrystallized on contact with the dissolution medium. There is no difference between the dissolution rate of amorphous/crystalline HTZ and the release rate of HTZ from coamorphous GLZ-HTZ. Crystalline HTZ and GLZ were identified in the XRPD pattern after the dissolution testing of coamorphous GLZ-HTZ (Fig. S17).

There is also no dissolution advantage for amorphous TRI, either as the pure amorphous phase or coamorphized with GLZ (Figs. 5b and S16). GLZ is released at a slower rate from GLZ-TRI compared to crystalline GLZ and again, this can be attributed to clumping and dispersibility problems. Solution-mediated recrystallization can be clearly ruled out as the reason for the unexpected poor dissolution properties of the coamorphous system, since XRPD measurements indicated that the GLZ-TRI sample remained amorphous during the dissolution study (Fig. S18). In this context it is noteworthy that - in contrast to GLZ-HTZ -
GLZ-TRI appears to be stabilized by drug-drug intermolecular interactions as suggested by the IR data.

Fig. 5. Dissolution profiles of coamorphous GLZ-HTZ, GLZ-TRI, crystalline GLZ, HTZ, and TRI. (a) ▲ release of GLZ from coamorphous GLZ-HTZ; □ release of HTZ from coamorphous GLZ-HTZ; ● crystalline HTZ; △ crystalline GLZ; (b) ▲ release of GLZ from co-amorphous GLZ-TRI; □ release of TRI from coamorphous GLZ-TRI; ○ crystalline TRI; △ crystalline GLZ.

As the lack of a dissolution advantage of coamorphous GLZ-TRI was attributed to poor dispersibility and wettability, the effect of surfactants was explored. GLZ and TRI were co-amorphized in the presence of sodium dodecyl sulfate (SDS) and sodium taurocholate (NaTC). The XRPD patterns of samples containing 15 wt% SDS showed crystalline GLZ after exposure to the dissolution medium for 2 h. The effect of excipients on the physical stability of coamorphous systems is little explored. Very recently, Ojarinta et al. described a decrease in the physical stability of coamorphous ibuprofen-arginine when xylitol was added as a diluent to a tablet formulation and attributed this to the hygroscopicity of the sugar alcohol (Ojarinta et al., 2018). Alternatively, residual crystal seeds of SDS may trigger heterogeneous nucleation in our case. Commercial NaTC is amorphous and in contrast to GLZ-TRI-SDS, samples containing 15 wt% NaTC remained X-ray amorphous. Hence,
dissolution studies were carried out on GLZ-TRI-NaTC (Fig. 6). A significantly larger percentage of TRI was released from coamorphous GLZ-TRI-NaTC compared to a physical mixture of crystalline GLZ, TRI and NaTC. For GLZ, no dissolution advantage was observed.

![Graph showing dissolution profiles](image)

**Fig. 6.** Dissolution profiles of coamorphous and crystalline GLZ-TRI-NaTC. (a) ▲ release of GLZ from coamorphous GLZ-TRI-NaTC; □ release of TRI from coamorphous GLZ-TRI-NaTC; ● release of TRI from a physical mixture of crystalline GLZ, crystalline TRI and NaTC; Δ release of GLZ from a physical mixture of crystalline GLZ, crystalline TRI and NaTC.

The dissolution profiles of crystalline and coamorphous (BZA⁺)(GLZ⁻) were also measured (Fig. 7). As expected, salt formation led to an enhanced dissolution rate. (BZA⁺)(GLZ⁻) has a relatively low melting point of 177.3 °C as determined by DSC indicating a low lattice energy which should be an important factor for the dissolution performance. On contact with the dissolution medium coamorphous (BZA⁺)(GLZ⁻) converts to the crystalline salt (Fig. S19). In addition, clumping is observed so that there is no dissolution advantage of the amorphous phase.
Fig. 7. Dissolution profiles of crystalline (BZA\(^+\))(GLZ\(^-\)) (□), amorphous (BZA\(^+\))(GLZ\(^-\)) (▲) and crystalline GLZ (●).

3.5 Solid-state forms of IND

Our findings on the stability of IND form I at high RH prompted us to investigate the solid-state forms of IND and their phase transformations in more detail. As already described above, neat milling of the commercial form leads to amorphization. No change of the XRPD pattern and IR spectrum is observed, when IND\(\cdot\)0.5\(\text{H}_2\text{O}\) is milled in the presence of water, except for a sharpening of the peaks (Fig. S20a and S21). Ghugare et al. obtained three solvates from solution (CCl\(_4\), C\(_6\)H\(_6\), Et\(_2\)O) (Ghugare et al., 2010). To explore the formation of further solvates or solvent-mediated solid-state transformations, we performed liquid-assisted milling with methanol, acetonitrile, acetone, ethanol, chloroform and dichloromethane (Figs. S20-22). Milling in the presence of ethanol, acetonitrile and acetone led to IND form I, while in the case of methanol a different IR spectrum and XRPD pattern A was observed. The broad \(v(\text{NH}_2)\) peaks of commercial IND (IND\(\cdot\)0.5\(\text{H}_2\text{O}\)) at 3287 and 3311 cm\(^{-1}\) are shifted to 3278 and 3321 cm\(^{-1}\) and have a higher intensity in A. The \(v(\text{C}=\text{O})\) vibration that appears at 1655 cm\(^{-1}\) in IND\(\cdot\)0.5\(\text{H}_2\text{O}\) is observed at 1673 cm\(^{-1}\) with a shoulder at 1644 cm\(^{-1}\). Differences in the \(v_\text{as}(\text{SO}_2)\) and \(v_\text{as}(\text{SO}_2)\) bands in the spectrum of commercial IND and A are <2 cm\(^{-1}\). The
changes are indicative of a change in the intermolecular H bonding interactions. A comparison with the XRPD pattern displayed in Fig. S9 shows that IND converts to the same new solid-state form as in the case of methanol-assisted grinding of GLZ-IND. The DSC plot of A revealed no thermal events other than melting at 181.9 ° (Fig. S23) so that it seems unlikely that form A is a methanol solvate. In line with this, the XRPD pattern did not change when A was heated for two weeks at 140 °C. Neither did the XRPD show any changes, when form A was kept at 98 % RH for four weeks. Unfortunately, all attempts to obtain X-ray suitable single crystals of form A were unsuccessful. A new pattern was obtained after milling IND-0.5H2O with CHCl3. The endotherm at 134.9 °C in the DSC combined with an 11 % weight loss in the thermogravimetric analysis (Fig. S24) is consistent with the formation of a solvate of composition IND-0.34CHCl3. Milling with CH2Cl2 gave an almost identical XRPD pattern suggesting an isomorphous CH2Cl2 solvate. At 98 % RH, both solvates transform to the hemihydrate IND-0.5H2O (Fig. S25). The solid-state transformations of IND-0.5H2O are summarized in Fig. 8.

**Fig. 8.** Milling- and heating-induced solid-state transformations of IND. LAG = liquid-assisted grinding.
Conclusions

Cocrystallization of strongly basic BZA with GLZ gives a low melting, rapidly dissolving salt. GLZ does not form cocrystals with the benzothiazides CTZ and HTZ, the pteridine TRI, the 1,4-dihydropyridine NIF or the sulfonamide IND. The crystal structure of GLZ shows the presence of the sulfonamide and amide dimer homosynthons (N-H···O=S and N-H···O=C dimers, R2̄(8) rings, Parvez et al., 1999) which are apparently preferred over the heterosynthons that could form in the potential cocrystals. On the other hand, GLZ easily amorphizes on milling with HTZ, TRI, IND, CTZ and NIF. Out of the five coamorphous systems GLZ-TRI (1:1) presents a pharmaceutically viable coamorphous formulation, as it is stable in air and dissolution medium, contains the two drugs in an appropriate ratio and gives an enhanced dissolution rate for TRI, when a suitable surfactant is added. Milling of IND-0.5H2O in the presence of traces of methanol produces a new (presumably solvent-free) solid-state form of IND.

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Appendix-A. Supplementary data

Tables with details of the coamorphization, cocrystallization and dissolution studies, H bonding interactions in (BZA+)(GLZ−) and IND-0.5H2O, selected bond lengths, bond angles and H bonding interactions in Na+GLZ−, additional XRPD patterns, calculated XRPD patterns, IR spectra, DSC plots.

References


Table 1. Crystallographic data of (BZA⁺)(GLZ⁻), Na⁺GLZ⁻, and IND·0.5H₂O

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<th>Na⁺GLZ⁻</th>
<th>IND·0.5H₂O</th>
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<td>C₁₆H₁₇ClN₃O₃S₅S</td>
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<td>345.39</td>
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<td>colorless block</td>
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<td>0.5 × 0.4 × 0.2</td>
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<td>30.0598(11)</td>
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<td>9.8387(5)</td>
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