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Research paper

# Dipeptides as co-formers in co-amorphous systems

Wenqi Wu[a], Korbinian Löbmann[a,*], Jan Schnitzkewitz[a], Astrid Knuhtsen[b], Daniel Sejer Pedersen[b], Thomas Rades[a,c], Holger Grohganz[a]

[a] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[b] Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
[c] Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

ABSTRACT

Drug-amino acid co-amorphous systems have become increasingly well-investigated systems to improve dissolution rate of poorly water-soluble drugs. In this study, dipeptides were investigated as co-formers for co-amorphous systems based on the hypothesis that dipeptides might combine the inherent properties of the two included amino acids. Co-amorphization of the model drug mebendazole was investigated with five dipeptides, tryptophan-phenylalanine, phenylalanine-tryptophan, aspartic acid-tyrosine, histidine-glycine and proline-tryptophan. The dipeptides were chosen to investigate whether the side chains (nonpolar, polar, basic and acidic), and the sequence of amino acids (tryptophan-phenylalanine versus phenylalanine-tryptophan) have an influence on the performance of dipeptides as co-formers. All mebendazole-dipeptide systems became amorphous after ball milling for only 30 min, while this generally was not the case for the single amino acids or physical mixtures of the amino acids forming the dipeptides. Dissolution studies showed that the dissolution rate of mebendazole from most co-amorphous systems was increased significantly compared to crystalline and amorphous mebendazole. However, no clear trend for the drug dissolution enhancement was observed within the different co-amorphous drug-dipeptide systems. The stability study revealed that co-amorphous mebendazole-dipeptide systems showed higher physical stability compared to amorphous mebendazole. In conclusion, dipeptides are shown to be promising co-formers for co-amorphous systems.

## 1. Introduction

The majority of drugs in current development and approximately half of the commercially available drugs show poor water solubility, and are therefore prone to low and variable bioavailability [1,2]. In the recent decades, various formulation strategies have been developed to address the poor aqueous solubility and slow dissolution rate challenge. Particle size reduction [3], and salt formation can be an attractive strategy [4,5]. Moreover, lipid-based drug delivery systems, such as self-(micro- or nano-) emulsifying drug delivery systems [6–8] or lipid solutions [9], are a promising approach to improve the poor water solubility and bioavailability of lipophilic drugs. Additionally, converting crystalline drugs into their amorphous form is one method to improve the water solubility of such drugs [10]. The energy in amorphous forms is higher than in their crystalline counterparts, leading to a higher dissolution rate and apparent solubility. However, for the same reason, amorphous forms are thermodynamically unstable and tend to recrystallize [11]. Thus, physical stabilisation of amorphous drugs is a major challenge.

In recent years, co-amorphous systems have attracted some attention as a promising formulation approach to address the poor water solubility of drugs. Co-amorphous systems usually consist of two or more, initially crystalline, low molecular weight compounds, which upon processing form a single homogenous amorphous phase. Some co-amorphous systems are drug-drug systems intended for fixed dose combination, such as indomethacin and ranitidine hydrochloride [12], naproxen and cimetidine [13] or tranilast and diphenhydramine hydrochloride [14]. Whilst this is a promising approach, especially if both drugs are poorly water-soluble [15], there are some limitations for a broader application. The drugs in combination should act by different pharmacological mechanisms and the molar doses should not be too different, since usually the 1:1 M ratio of the binary co-amorphous mixture is the most stable [15]. In addition, the pharmacokinetics of the drugs must be taken into consideration as a synchronized release for the co-amorphous drugs at the 1:1 ratio might occur [13].

Subsequently, drug-excipient systems with a more general applicability were developed. The initial focus of these systems was on drug-amino acid mixtures [16]. After successfully using arginine as co-former [16–18], various other co-amorphous systems based on different amino acids have been investigated [19–21]. The dissolution rate of co-amorphous systems was significantly higher compared to that of the respective crystalline and pure amorphous drugs, and physical stability was also higher than that of the pure amorphous drugs [16]. Over the last years, other small molecular weight co-formers, such as organic acids [22,23], saccharin [24], and bile salts [25] have also been investigated.

* Corresponding author.
*E-mail address:* korbinian.loebmann@sund.ku.dk (K. Löbmann).

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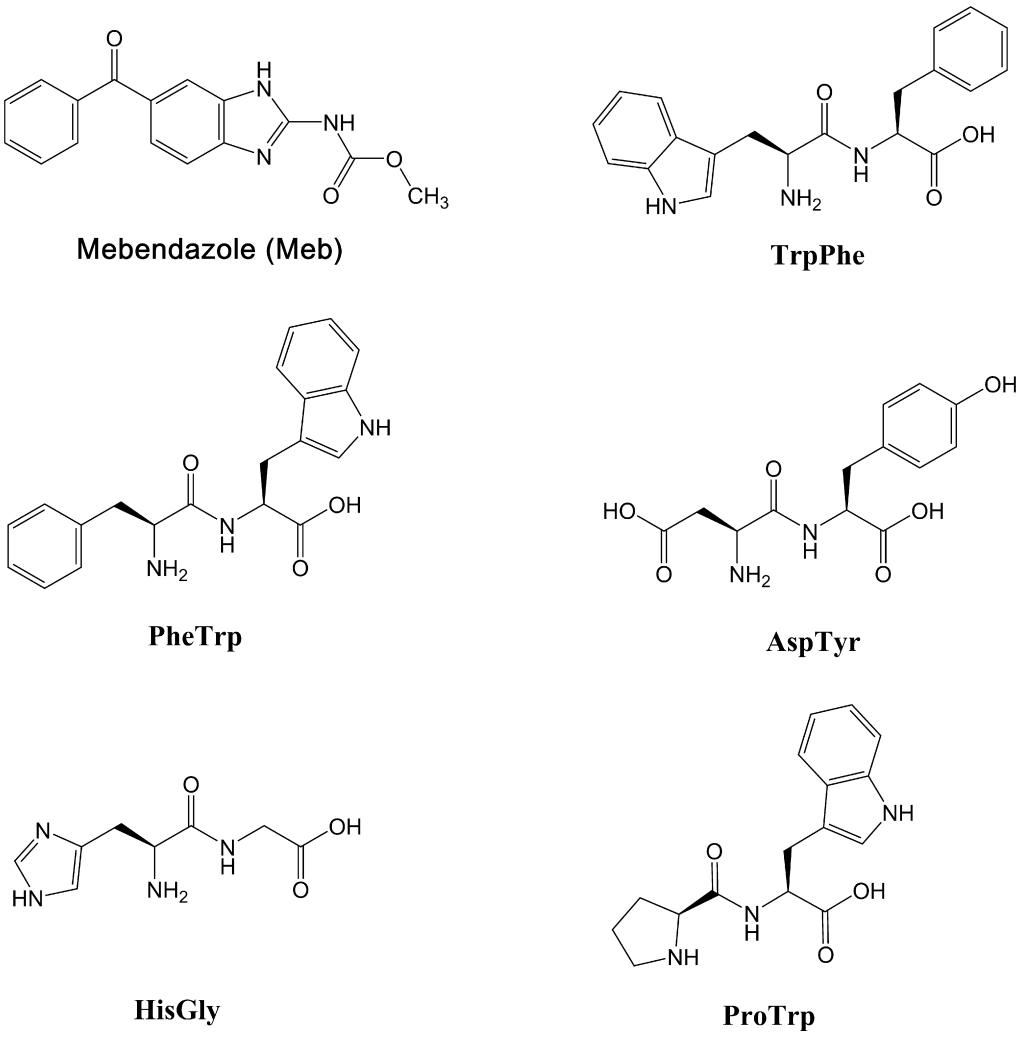


**Fig. 1.** Chemical structures of mebendazole and the dipeptides used in this study.

Among all these possibilities, amino acids remain important and promising co-formers, due to their variable chemical side chain structures, which result in variable physical-chemical properties and possibilities of drug-amino acid interaction. For instance, a basic side chain may interact with an acidic functional group of a given drug, and the potential resulting salt formation can be a key point to form stable co-amorphous systems [26,27]. Other molecular interactions between the amino acids side chains and drugs, such as π–π interactions [17] and hydrogen bonding [28] have also been shown to play important roles in the formation of homogeneous co-amorphous systems.

However, some issues remain unaddressed. For example, it is a challenge for most polar and acidic amino acids to form co-amorphous systems with given drugs, while non-polar amino acids, such as tryptophan and phenylalanine, and basic amino acids (arginine, histidine and lysine) easily form co-amorphous systems [20,29]. It should also be noted that the formation of a co-amorphous system does not necessarily lead to a high increase in the drug dissolution rate. For instance, co-amorphous carbamazepine-tryptophan showed excellent physical stability but the dissolution rate of the drug was similar to that of crystalline carbamazepine [16]. On the other hand, other amino acids, such as proline, lead to an increased dissolution rate and solubility [30], but showed unsatisfactory long-term physical stability [29].

It is hypothesized in this study that the combination of two amino acids in the form of a dipeptide may result in a combined effect, i.e. easy formation of co-amorphous system with a given drug with satisfactory physical stability, as well as an increased dissolution rate. This is supported by a recent study in which the dipeptide-related compound aspartame, a methyl ester of the aspartic acid/phenylalanine dipeptide, showed to be superior to the respective single amino acids with regard to formation of a co-amorphous systems and dissolution rate improvement [31]. The obvious question to be addressed following this hypothesis is which role the combination of various amino acids will play for the properties of the co-amorphous system. Specifically, it is of interest to evaluate the effect of amino acid side chain types (polar, non-polar, acidic, basic) and amino acid sequence (amino acid A-amino acid B versus amino acid B-amino acid A). Therefore, five dipeptides were chosen for the current study: tryptophan-phenylalanine (non-polar-nonpolar, A-B), phenylalanine-tryptophan (nonpolar-nonpolar, B-A), aspartic acid-tyrosine (acidic-polar), histidine-glycine (basic-non-polar) and proline-tryptophan (polar-nonpolar). Mebendazole (a biopharmaceutics classification system (BCS) class II basic drug [32]) was used as a model drug based on previous experience with single amino acids and aspartame [31]. Mebendazole was ball milled with the single amino acids, the various dipeptides and the corresponding amino acid mixtures in order to prepare co-amorphous samples. X-ray powder diffraction (XRPD) and modulated temperature differential scanning calorimetry (mDSC) were used to characterize the solid state of the samples. Powder dissolution and physical stability studies were conducted to evaluate the pharmaceutical performance of the dipeptides as co-formers.

## 2. Materials and methods

### 2.1. Materials

Mebendazole (Meb, MW = 295.29 g/mol), L-aspartic acid (Asp, MW = 133.10 g/mol), L-tyrosine (Tyr, MW = 181.19 g/mol), L-histine (His, MW = 155.16 g/mol), L-glycine (Gly, MW = 75.07 g/mol), L-proline (Pro, MW = 115.13 g/mol), L-tryptophan (Trp, MW = 204.23 g/mol), L-phenylalanine (Phe, MW = 165.19 g/mol), methanol and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, USA). All the dipeptides, tryptophan-phenylalanine (TrpPhe), phenylalanine-tryptophan (PheTrp), aspartic acid-tyrosine (AspTyr), histidine-glycine (HisGly) and proline-tryptophan (ProTrp) were obtained from GL Biochem (Shanghai) Ltd (Shanghai, China). For a better understanding, the notations Asp-Tyr, His-Gly, Trp-Phe, Phe-Trp and Pro-Trp were used to describe the physical mixtures of two individual amino acids. All compounds were used as received. The chemical structures of the dipeptides and mebendazole are shown in Fig. 1.

### 2.2. Sample preparation

Vibrational ball milling (BM) was used to prepare pure amorphous Meb, Meb-dipeptide (molar ratio of 1:1) and Meb-amino acid mixtures (molar ratio of 1:1:1). A total mass of 500 mg was added into 25 mL ball milling jars with two 12 mm stainless steel balls, and then ball milled in an oscillatory ball mill (Mixermill MM400, Retsch GmbH & Co., Haan, Germany) at 30 Hz in a cold lab (5 °C). The ball milling jars were sealed with parafilm to prevent moisture absorption during the BM process. Amorphization of the samples was characterized by XRPD (Section 2.3) after different BM times (30 min, 60 min, 90 min). Samples were ball milled for up to 180 min, if complete amorphization was not observed within 90 min. All samples were stored in a desiccator under vacuum at −20 °C until analysis to avoid moisture absorption. The samples used for modulated temperature differential scanning calorimetry analysis, dissolution and physical stability study were milled for 90 min.

### 2.3. X-ray powder diffraction (XRPD)

The solid state of the samples was analysed by XRPD. An X'Pert PANalytical X'Pert PRO X-ray Diffractometer (PANalytical, Almelo, The Netherlands) with Cu Kα radiation (λ = 1.54187 Å) was used to perform the measurements. The acceleration voltage and current were setup to 45 kV and 40 mA, respectively. Samples were scanned in reflection mode from 5° to 30° 2θ at a rate of 0.067° 2θ/s and a step size of 0.026° 2θ. The data was collected and analysed with an X'Pert Data Collector (PANalytical, Almelo, The Netherlands).

### 2.4. Modulated temperature differential scanning calorimetry (mDSC)

The mDSC thermograms of the ball milled samples were collected using a Discovery DSC (TA instruments, New Castle, USA). Routine calibration of the instrument was performed with an indium standard. The nitrogen gas flow was 50 mL/min. Approximately 4 mg (2–6 mg) samples were filled in aluminium Tzero pans and smoothed using a cylinder to ensure a flat layer and a good contact with the sample pan. Then the samples were sealed with a hermetic lid to avoid possible moisture absorption. The measurements were conducted in modulated temperature mode: after 5 min of an isothermal step at −10 °C, samples were heated to 180 °C at a heating rate of 2 °C/min, amplitude of 0.212 °C and period of 40 s. The DSC data was analysed using TA instruments Trios software (version 3.3.1). The glass transition temperatures ($T_g$, midpoint) were determined from the reversing heat flow signal. Each sample was measured in triplicate and reported as mean ± standard deviation.

### 2.5. Powder dissolution studies

#### 2.5.1. Dissolution test

Powder dissolution tests were performed in 100 mL pH 6.8 phosphate buffer (0.1 M) at 37 ± 0.5 °C with an ERWEKA DT70 dissolution tester (Heusenstamm, Germany). A custom-made, down-scaled version of USP apparatus 2 with a vessel size of 250 mL was applied due to the small amount of dissolution medium. The apparatus is similar to the standard USP apparatus 2, with a similar water bath, heater, control panel, etc., but equipped with mini vessels, specific vessel holders and mini paddles. The mini vessels are miniaturized from the standard USP vessels, and the mini paddles are based on the USP paddle setup but scaled down 1/3 with respect to all dimensions, resulting in essentially similar hydrodynamics as the standard USP apparatus 2 [33]. Since the saturation concentration of Meb in the pH 6.8 phosphate buffer is very low (0.64 μg/mL), a mass of 5 mg Meb or Meb-co-former mixtures containing 5 mg of Meb were placed in the dissolution medium to perform the non-sink condition dissolution test. The paddles rotated at 100 rpm. Samples (1 mL) were withdrawn at predetermined time points (2, 5, 10, 20, 30, 60, 120, 180, 240, 300, 360 and 1440 min), and replaced with 1 mL pre-warmed dissolution buffer. The samples were filtered (0.45 μm), and diluted with acetonitrile at a 1:1 vol ratio to avoid potential precipitation of Meb from supersaturated samples. The diluted samples were quantified by HPLC. All dissolution experiments were performed in triplicate.

#### 2.5.2. HPLC analysis

All samples from the dissolution studies were analysed on a Dionex HPLC system (Germering, Germany) equipped with a P680 pump, an ASI-100 automated sample injector and a PDA 100 photodiode array detector. A reverse-phase C18 column (Phenomenex, 100 × 4.6 mm) and UV detection at 312 nm were used, with a constant flow rate of 1.0 mL/min and an isocratic solvent system. The mobile phase consisted of 47% 0.05 M $KH_2PO_4$, 20% acetonitrile and 33% methanol (v/v), as described in a previous study [34]. The resulting retention time was 3.31 ± 0.02 min. The injection volume was 20 μL, and chromatograms were analysed by using Chromeleon Version 7.1.3.2425 software.

### 2.6. Physical stability

Amorphous Meb and co-amorphous samples were stored in desiccators over silica gel at 40 °C and 25 °C. The relative humidity was around 2%, monitored with EasyLog EL-USB-2 sensors (Lascar Electronics, Essex, England). The recrystallization tendency was analysed by XRPD 1, 2, 3, and 4 weeks, and then 2 and 3 months after sample preparation.

## 3. Results and discussion

### 3.1. Formation of co-amorphous systems

After BM, the powders were evaluated by XRPD to determine whether the pure Meb, Meb-dipeptide and Meb-amino acid(s) binary and ternary mixtures became amorphous. The results are shown in Fig. 2. The milling time needed for complete amorphization was investigated and is shown in Table 1 to indicate the ease of amorphization of the samples.

From Fig. 2 and Table 1, it can be seen that it was possible to convert Meb alone into an amorphous form; however a ball milling time of 90 min was required. In the presence of a dipeptide as co-former, a reduction of the milling time down to 30 min to obtain an amorphous product was observed, indicating the promising applicability of dipeptides as co-formers, since a short ball milling time needed for complete amorphization indicates good co-formability of the co-formers [29]. In contrast, the use of single amino acids or mixtures thereof was less successful in obtaining an amorphous system. The only

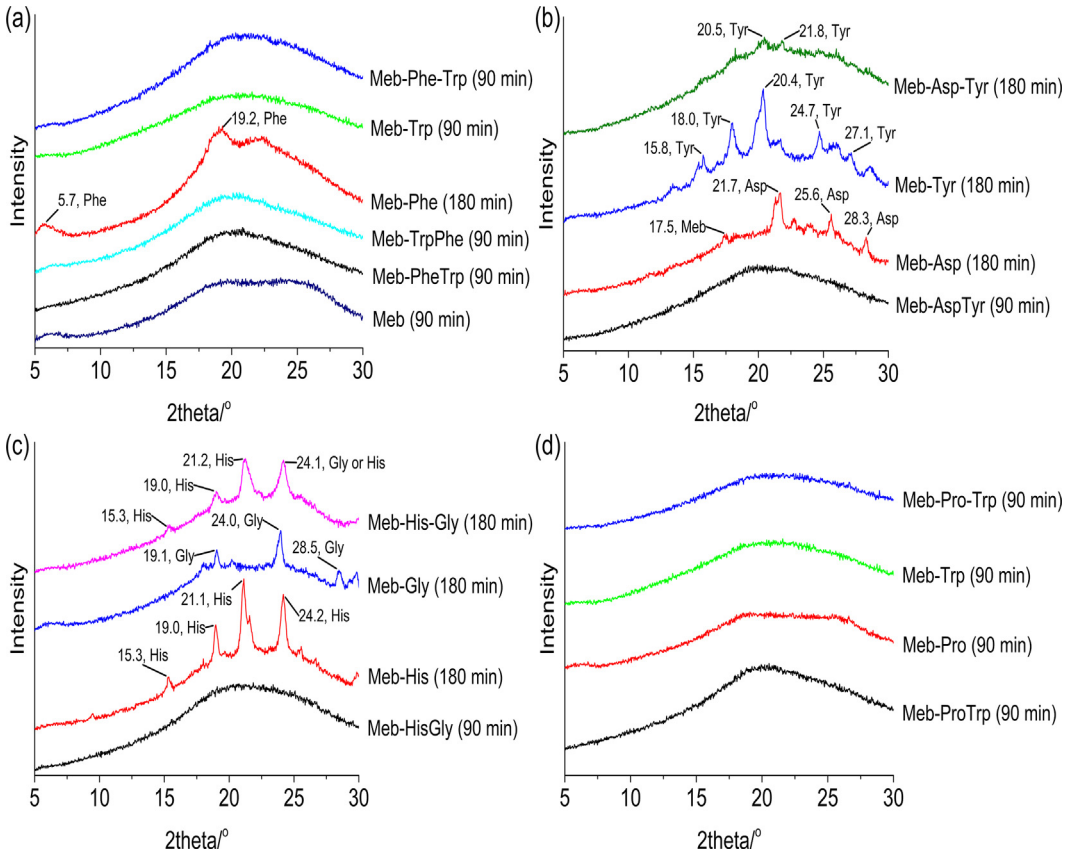


**Fig. 2.** XRPD diffractograms of (a) pure Meb, Meb-TrpPhe, MEB-PheTrp and individual Meb-amino acid binary/ternary mixtures, (b) Meb-AspTyr and individual Meb-amino acid binary/ternary mixtures, (c) Meb-HisGly and individual Meb-amino acid binary/ternary mixtures and (d) Meb-ProTrp and individual Meb-amino acid binary/ternary mixtures after BM (milling time stated in brackets in the figure legends).

**Table 1**
Success in amorphization after ball milling and the origin of remaining diffraction peaks.

| Sample content | Molar ratio | Milling time (min) | Success in amorphization (yes/no) | Origin of remaining diffraction peaks |
|---|---|---|---|---|
| Meb | – | 90 | Yes | – |
| Meb-TrpPhe | 1:1 | 30 | Yes | – |
| Meb-PheTrp | 1:1 | 30 | Yes | – |
| Meb-AspTyr | 1:1 | 30 | Yes | – |
| Meb-HisGly | 1:1 | 30 | Yes | – |
| Meb-ProTrp | 1:1 | 30 | Yes | – |
| Meb-Phe | 1:1 | 180 | No | Phe |
| Meb-Phe-Trp | 1:1:1 | 90 | Yes | – |
| Meb-Asp | 1:1 | 180 | No | Asp |
| Meb-Tyr | 1:1 | 180 | No | Tyr |
| Meb-Asp-Tyr | 1:1:1 | 180 | No | Asp and Tyr |
| Meb-His | 1:1 | 180 | No | His |
| Meb-Gly | 1:1 | 180 | No | Gly |
| Meb-His-Gly | 1:1:1 | 180 | No | His and Gly |
| Meb-Pro | 1:1 | 60 | Yes | – |
| Meb-Trp | 1:1 | 30 | Yes | – |
| Meb-Pro-Trp | 1:1:1 | 30 | Yes | – |

successful amorphous formulations were obtained with Trp, Pro-Trp, Pro and Phe-Trp after 30 min, 30 min, 60 min and 90 min BM, respectively. All other investigated binary and ternary mixtures showed remaining crystallinity of the amino acids even after BM for up to 180 min. The co-formers that could not form an amorphous phase with Meb after 180 min BM (Phe, Asp, Tyr, His, Gly, amino acid physical mixtures: Asp-Tyr and His-Gly), were defined as poor co-formers (poor co-formability) in this study; the co-formers that formed an amorphous phase with Meb within 30 min of BM were defined as good co-formers (good co-formability): all the five dipeptides, Trp, and the physical mixture of Pro and Trp (Pro-Trp).

From the XRPD study, it becomes evident that the actual use of a dipeptide in itself is the crucial factor for the improved amorphization rather than merely the properties of the side chains, since all Meb-dipeptide systems became amorphous, while only Meb-Phe-Trp and Meb-Pro-Trp amorphized successfully when the amino acid physical mixtures were used as co-formers. One of the possible reasons why the dipeptides appear to be superior to single amino acids in relation to inducing amorphization could be the higher molecular weight of dipeptides compared to the single amino acids. A previous study has shown that compounds with higher molecular weight can be converted to amorphous forms easier than compounds with lower molecular weight [35]. In addition, the rigidity of the molecules may also play a role in the amorphization process; higher rigidity leads to low configurational entropy and thus facilitates crystallization from the amorphous state [36]. The larger dipeptide molecules are more flexible than the smaller corresponding single amino acids, which therefore would show a higher tendency for amorphization and lower tendency to crystallization from the amorphous form. A specific influence on co-formability of the types of the amino acids (polar, non-polar, acidic and basic) in the dipeptides was not observed. This can be seen by the fact that the dipeptide of two amino acids which individually were characterized as poor co-formers (such as Asp and Tyr, His and Gly) showed a successful amorphization with Meb when being part of a dipeptide (AspTyr and HisGly). Thus, including at least one amino acid which is deemed a good co-former in the dipeptide when designing dipeptides as co-formers is not a prerequisite for a successful amorphization with given drugs. In addition, the amino acid sequence of the dipeptides also showed no effect on the amorphization process, as both Meb-TrpPhe

**Table 2**
Glass transition temperature of the amorphized samples after BM according to mDSC measurements ($n$ = 3).

| Samples prepared by ball milling | The first $T_g$ ± standard deviation/the second $T_g$ ± standard deviation (°C) |
|---|---|
| Meb | 112.5 ± 0.3 |
| Meb-TrpPhe | 107.5 ± 0.2 |
| Meb-PheTrp | 104.6 ± 0.2 |
| Meb-AspTyr | 61.2 ± 0.9 |
| Meb-HisGly | 34.9 ± 1.2/89 ± 0.6 |
| Meb-ProTrp | 60.5 ± 0.2 |
| Meb-Trp | 128.7 ± 0.2 |
| Meb-Pro | 96.9 ± 0.1 |
| Meb-Pro-Trp | 56.3 ± 0.2 |
| Meb-Trp-Phe | 119.0 ± 0.1 |

and Meb-PheTrp formed an amorphous phase within 30 min BM. As discussed above, one of the reasons to explain why amino acid types and sequence of the dipeptides showed no specific effect on the amorphization could be attributed to the molecular weight and rigidity of the co-formers.

In conclusion, the results shown in Fig. 2 and Table 1 support the hypothesis that dipeptides are promising co-formers and somewhat superior in the formation of an amorphous system compared to single amino acids and amino acids physical mixtures.

### 3.2. Modulate temperature differential scanning calorimetry (mDSC)

The successfully amorphized systems were further analysed using mDSC measurements to identify whether the Meb-co-former combinations were truly co-amorphous, i.e. forming a homogeneous amorphous single phase (characterized by a single $T_g$), or were a physical mixture of two amorphous phases, as indicated by several $T_g$s. The glass transition temperatures of the obtained amorphous samples after BM are summarized in Table 2 and the DSC thermograms are shown in Fig. 3.

Amorphous Meb had a $T_g$ at 114.5 °C (Table 2). All co-amorphous systems showed only a single $T_g$, except Meb-HisGly, for which two $T_g$s were observed. As discussed above, one $T_g$ usually indicates presence of a single homogeneous phase, while two $T_g$s indicate two presence of two amorphous phases. For the binary and ternary amorphous mixtures of Meb with amino acids, also a single $T_g$ was found indicating the formation of a co-amorphous system.

The sequence of amino acids in dipeptides, like TrpPhe and PheTrp, showed no influence on the formation of a co-amorphous system with Meb: both Meb-TrpPhe and Meb-PheTrp became amorphous after BM 30 min and showed a similar $T_g$. An interesting observation was made with regards to the $T_g$s of Meb-dipeptide co-amorphous systems compared with the ternary Meb-amino acids systems. The $T_g$ of Meb-TrpPhe, Meb-PheTrp, as well as Meb-ProTrp, was similar to the $T_g$ of the corresponding Meb-Trp-Phe and Meb-Pro-Trp ternary systems, indicating that there was no significant difference with respect to the $T_g$ when the dipeptides or the mixtures of individual amino acids were used as co-formers.

### 3.3. Powder dissolution studies

Powder dissolution was conducted on the different co-amorphous formulations and their dissolution performance and potential to supersaturate was compared to the dissolution performance of crystalline and amorphous Meb (Fig. 4).

Crystalline Meb showed the lowest dissolution rate among all investigated samples (Fig. 4); the concentration of dissolved Meb increased slowly and achieved approximately saturation concentration of

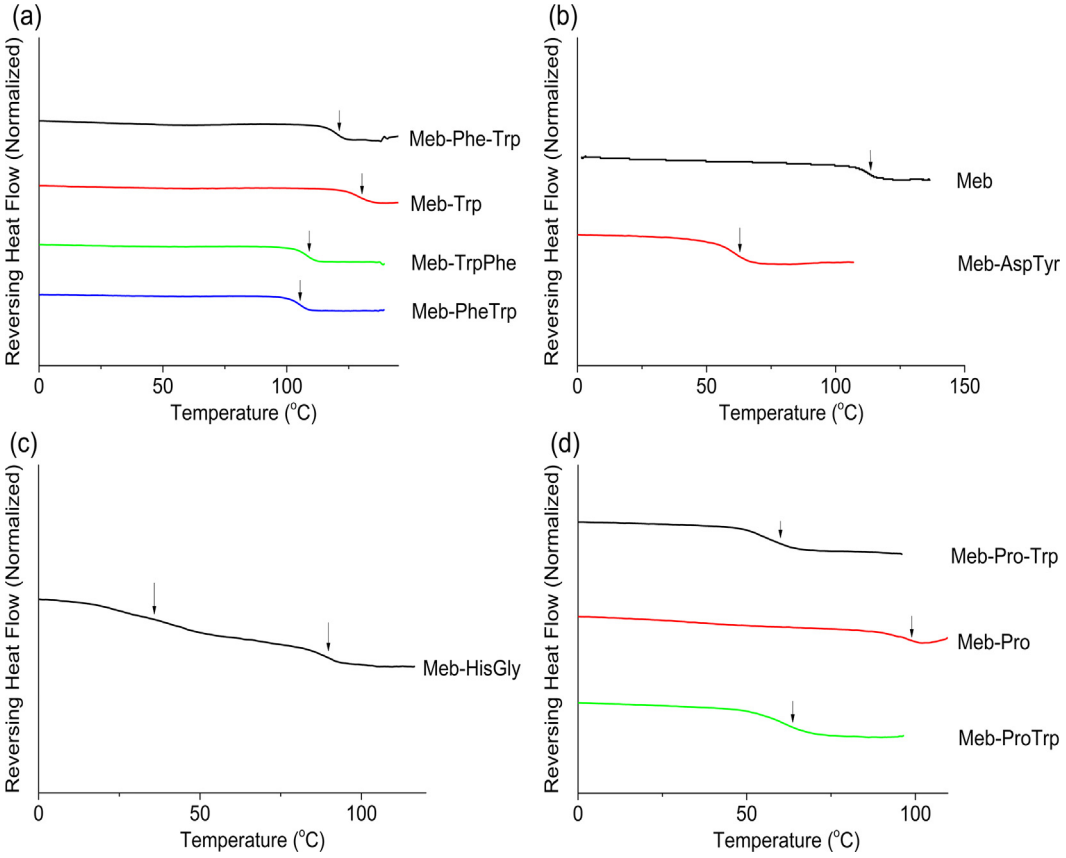


**Fig. 3.** mDSC thermograms of amorphized (a) Meb-Phe-Trp, Meb-Trp, Meb-TrpPhe and Meb-PheTrp, (b) pure Meb and Meb-AspTyr, (c) Meb-HisGly and (d) Meb-Pro-Trp, Meb-Pro and Meb-ProTrp.

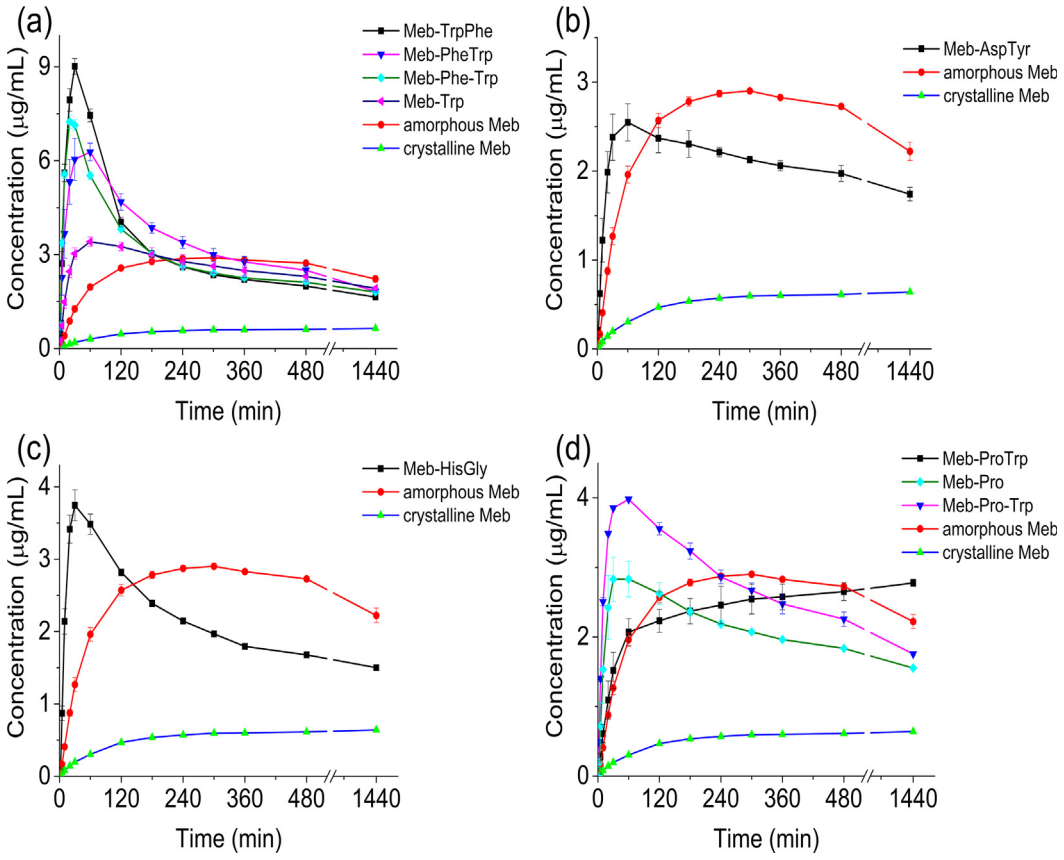


**Fig. 4.** Powder dissolution profiles of crystalline Meb, amorphous Meb and the co-amorphous Meb-co-former systems.

the crystalline form after 1440 min. In contrast, amorphous Meb showed a much faster dissolution rate in the first 120 min of dissolution. A notable supersaturation was observed in amorphous Meb, as the concentration increased to a $C_{max}$ of 2.90 μg/mL at 300 min, followed by a slight decrease to 2.22 μg/mL after 1440 min due to precipitation of the drug from the supersaturated state. The majority of the co-amorphous systems showed slightly higher dissolution rates (Meb-AspTyr, Meb-HisGly, Meb-Trp, Meb-Pro-Trp, Meb-Pro) compared to amorphous Meb, with exception of Meb-ProTrp, which showed a similar dissolution rate as amorphous Meb, and the samples containing Trp and Phe in the form of co-amorphous Meb-TrpPhe, Meb-PheTrp, Meb-Phe-Trp, which showed a much higher degree of dissolution rate (Fig. 4a). It is worthy to mention that the particle size reduction due to milling may have contributed to the dissolution rate improvement of the amorphous formulations. However, the pure amorphous drug was also prepared by ball milling under the same conditions as the amorphous samples containing either amino acids or dipeptides. Hence, the similar preparation makes a comparison of the co-amorphous formulations to the pure amorphous drug meaningful. For instance, co-amorphous Meb-TrpPhe achieved a $C_{max}$ of 9.02 μg/mL after 30 min, which is approximately 46-fold and 7-fold higher than the drug concentration of crystalline and amorphous Meb after 30 min, respectively. Following supersaturation, the drug subsequently started to precipitate towards a concentration level of the $C_{max}$ of the pure amorphous drug at approximately 300 min, after which the precipitation kinetics slowed down and the concentrations remained similar to those obtained for pure amorphous Meb.

The dissolution behaviours of Meb-TrpPhe and Meb-PheTrp were similar, with only small differences at the start of the dissolution experiment and a higher supersaturation for Meb-TrpPhe. This observation agrees well with the XRPD and mDSC studies, in which Meb-TrpPhe and Meb-PheTrp showed similar co-amorphous formability and $T_g$. Comparing the dissolution behaviour of Meb-Trp and Meb-Phe-Trp showed a higher dissolution rate and a higher degree of supersaturation for Meb from the ternary system, indicating that the addition of Phe was beneficial for the dissolution rate and solubility enhancement. Considering that the water solubility of Phe is 2.6-fold higher than that of Trp [37], this is in agreement with a previous study, which showed that the addition of higher water-soluble co-former (proline) improved the dissolution rate of the drug [30].

Compared to Meb-PheTrp and Meb-TrpPhe systems, Meb-HisGly and Meb-AspTyr showed a lower degree of supersaturation, however in a similar range with each other. Meb-HisGly showed a slightly higher degree of supersaturation than Meb-AspTyr (Fig. 4b,c): Meb-HisGly achieved a $C_{max}$ (3.74 μg/mL) after 30 min, while Meb-AspTyr achieved a $C_{max}$ of 2.55 μg/mL after 60 min. Compared to amorphous and crystalline Meb, the dissolution rate of Meb-AspTyr and Meb-HisGly was significantly increased: for instance, the drug concentration of Meb-HisGly was approximately 3-fold higher than for amorphous Meb and 19-fold higher than for crystalline Meb after 30 min of dissolution testing. Subsequently, the Meb concentration started to decrease towards a concentration of 1.74 μg/mL and 1.50 μg/mL after 1440 min, respectively, which were lower than that of amorphous Meb (2.22 μg/mL). Considering the amino acid types included in AspTyr (acidic amino acid-polar amino acids) and HisGly (basic amino acid-nonpolar amino acid), no clear evidence of amino acid types included in dipeptides on the dissolution enhancement performance was found.

The dissolution rate of co-amorphous Meb-ProTrp was the slowest among all investigated dipeptides (Fig. 4d), and even slower than amorphous Meb. Additionally, no supersaturation was observed in the dissolution profile of co-amorphous Meb-ProTrp. Although ProTrp consists of two non-polar amino acids, similar to TrpPhe and PheTrp, the dissolution enhancement of ProTrp was obviously different from that of TrpPhe and PheTrp, indicating that the amino acids types are

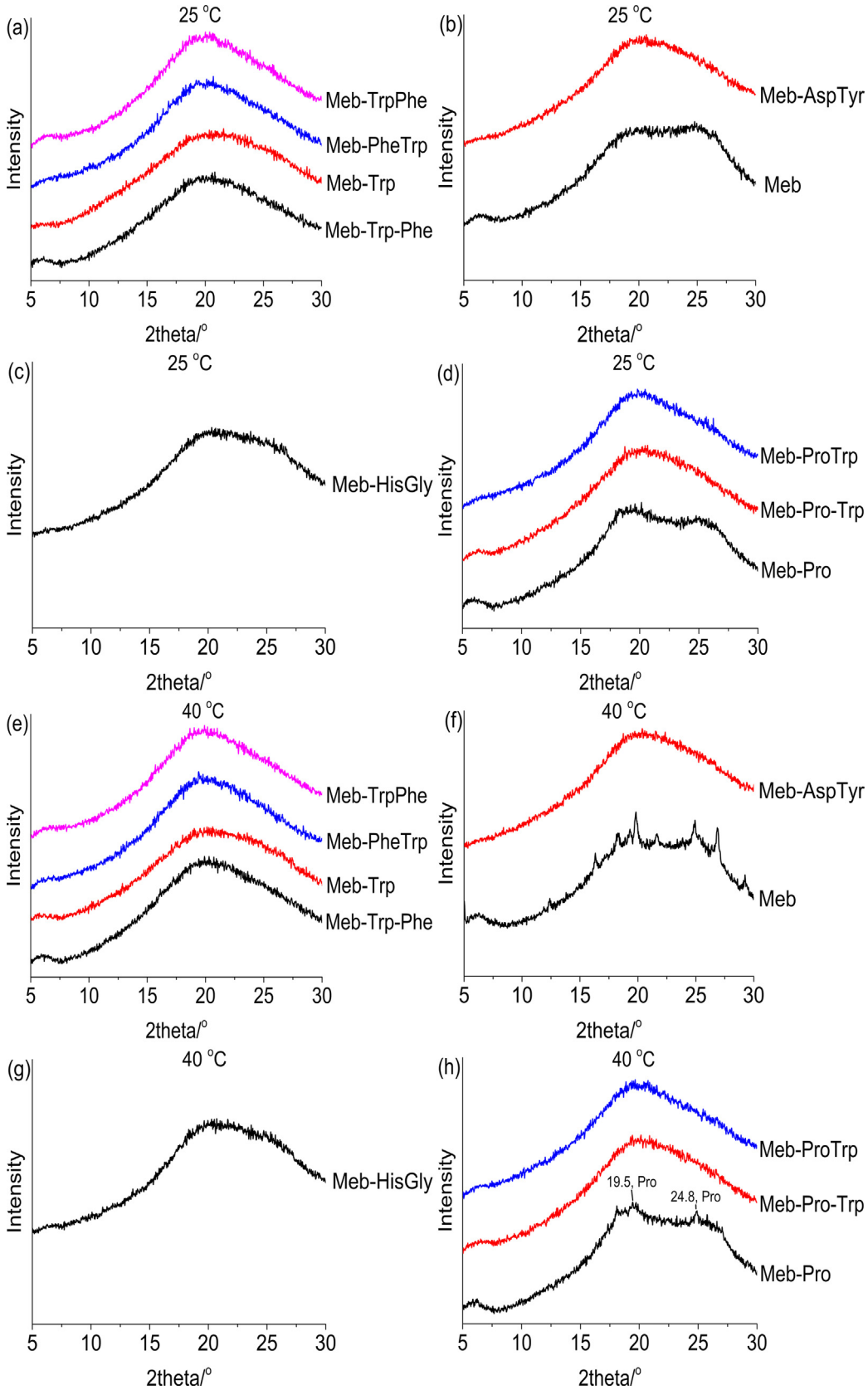


**Fig. 5.** X-ray powder diffractograms of pure amorphous Meb and co-amorphous Meb-co-former systems after 3 months storage at 25 °C (a–d) and 40 °C (e–h) under dry condition.

not the key parameter in the dissolution rate improvement of Meb via dipeptide co-amorphization. However, the drug concentration of Meb-ProTrp continually increased during the whole dissolution process, achieving 2.78 μg/mL after 1440 min, 4.3-fold higher than for crystalline Meb. This was the highest drug concentration in all investigated systems after 1440 min of dissolution. The unique property of maintaining a high drug concentration for at least 1440 min should be further studied and possibly used in future formulation development applications.

In conclusion, all the investigated dipeptides increased the dissolution rate and water solubility of crystalline Meb, but with different profiles and to different degrees. This could be useful for different formulation purposes, for example, tailoring the dissolution behaviour according to the desired release behaviour for best pharmaceutical performance. However, a clear influence of the amino acid types included in the dipeptides on the dissolution performance could not be identified.

### 3.4. Physical stability

Besides dissolution enhancement, the physical stability of co-amorphous systems is another important property that needs to be considered for an improved pharmaceutical performance of a co-amorphous system. As shown in Section 3.2, the $T_g$s of Meb-dipeptide systems were lower than that of pure amorphous Meb. However, previous studies have shown that the $T_g$ is not always a good predictor for the physical stability of amorphous systems [38,39]. The physical stability of pure amorphous Meb and Meb-co-former co-amorphous systems were therefore investigated after storage at 25 °C and 40 °C under dry conditions (Fig. 5).

At 25 °C, all samples, including amorphous Meb, remained amorphous. However, differences in stability were observed at 40 °C. Pure amorphous Meb recrystallized within 3 months, while all Meb-dipeptide co-amorphous systems remained amorphous. This points to a superior stability compared to Meb-amino acid co-amorphous systems, since Meb-Pro recrystallized during storage. In conclusion, dipeptides used as co-formers improved the physical stability of amorphous Meb.

### 3.5. Comparison of dipeptides and amino acids as co-formers

Combining all the results above, it can be seen that the investigated dipeptides formed amorphous systems with Meb easier than most of the single amino acids and amino acid mixtures. A specific effect of the amino acid types included in the dipeptides on the amorphization of Meb-dipeptide was not observed. The use of dipeptides themselves, rather than the amino acids types plays the key role in amorphization, since all Meb-dipeptide mixtures became amorphous after 30 min of BM. Similarly, the dissolution studies showed no clear relationship between the dissolution enhancement of Meb-dipeptide systems and the amino acids types included in the dipeptides. This is exemplified by TrpPro which was expected to be a promising co-former according to our former assumption, since it combines a good co-former (Trp) [29] and a dissolution enhancer (Pro) [30]. However, neither significant dissolution enhancement nor supersaturation was obtained for Meb-TrpPro. Thus, whilst dipeptides showed good co-formability in general, their dissolution enhancement behaviour is more complicated and as yet unpredictable. Further investigations are necessary to understand the relationship between the dissolution enhancement with the amino acid types in dipeptides.

## 4. Conclusion

In this study, the feasibility of different types of dipeptides as co-formers for co-amorphous systems was investigated. The influence of the types (polar, non-polar, acidic, basic) and sequence (A-B and B-A) of amino acids included in the dipeptides on the performance of dipeptides as co-formers was compared with the performance of single amino acids and amino acid mixtures. Several conclusions can be made from the study. Firstly, in contrast to the individual amino acids, all dipeptides led to the formation of amorphous systems, whereof four dipeptides (exception: HisGly) formed a homogeneous co-amorphous system with Meb after only 30 min of BM. Secondly, all five dipeptides improved the dissolution rate and water solubility of Meb, although to varying degrees and with different profiles. Thirdly, dipeptides as co-formers improved the physical stability of amorphous Meb. The sequence of the amino acids in the dipeptides showed no effect on the amorphization property, dissolution behaviour and physical stability. In conclusion, dipeptides proved their potential as an additional option as co-formers for co-amorphous systems.

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