Formulating poorly water soluble drugs in co-amorphous drug systems using amino acids: challenges and possibilities

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List of Abbreviations

AA	Amino acid(s)
API	Active pharmaceutical ingredient
Arg	L-Arginine
Asp	L-Aspartic acid
ATR-FTIR	attenuated total reflection Fourier-transform infrared spectroscopy
BCS	Biopharmaceutics Classification System
BM	Ball milling, ball milled
Car	Carvedilol
DMSO	dimethyl sulfoxide
DSC	Differential scanning calorimetry, differential scanning calorimeter
DVS	Dynamic vapour sorption analysis
FD	Freeze drying, freeze dried
FDA	Food and Drug Administration, *United States federal agency acting as the health authority*
FTIR	Fourier transform infrared spectroscopy
Glu	L-Glutamic acid
GT	Gordon-Taylor equation
IDR	intrinsic dissolution rate
IR	Immediate release
MQ	Melt quenching, melt quenched
NIR	Near infrared
PASD	polymeric amorphous solid dispersions
Ph.Eur.	*Pharmacopeia Europaea*, European Pharmacopeia
Phe	L-Phenylalanine
PM	Physical mixture

PVP/VA	Polyvinylpyrrolidone/Vinyl acetate
RH	Relative humidity
SD	Spray drying, spray dried
ssNMR	Solid state nuclear magnetic resonance
T_g	Glass transition temperature
TPS	Terahertz pulsed spectroscopy
Trp	L-Tryptophan
XRPD	X-ray powder diffractometry

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Abstract

Eiman Atiek, *Formulating poorly water soluble drugs in co-amorphous drug systems using amino acids: challenges and possibilities*, 2018.

Poor water-soluble drugs remain a challenge in the development of medicines. Many strategies exist to increase the solubility of a drug, one of them consisting in co-amorphisation of the API with an amino acid. The use of amino acids as amorphisation co-formers may increase the success of amorphisation and enhance the physical stability of the mixture.

Carvedilol was used for this research project as a representative drug candidate from the BCS class II. Firstly, the study focused on the amorphisation ability of carvedilol with different amorphisation methods, melt quenching (MQ), freeze drying (FD) and ball milling (BM). In addition, the advantages and limitations of each method are evaluated. Secondly, the co-amorphous mixtures obtained were characterised using a range of analytical techniques (XRPD, DSC, NMR, FTIR, etc.). Finally, the dissolution profile of a co-amorphous tablet formulation was determined.

None of the tested amorphisation methods can be regarded as ideal. The three methods were classified in terms of flexibility in the choice of substances to be amorphised, feasibility of the method and physical stability of the resulting amorphous substances. Co-amorphisation by ball milling was successful only with neutral amino acids, although the mixtures exhibited only weak intermolecular interactions. Moreover, the amorphisation via freeze drying with aspartic acid was successful and formed a co-amorphous salt with carvedilol. Thermal behaviour analysis showed that the freeze-dried mixtures are more physically stable under heating than ball-milled mixtures, which showed a systematic recrystallisation above the glass transition temperature (T_g). The T_g provided useful insight on the amino acid selection and the optimal molar ratio with carvedilol. The hygroscopicity of the starting crystalline material prior to co-amorphisation affected the success of amorphisation negatively. Co-amorphisation of carvedilol with the selected amino acids decreased the apparent aqueous solubility of carvedilol. However, the intrinsic dissolution rate (IDR) was systematically higher in the co-amorphous form for the corresponding crystalline physical mixtures, which in turn exhibited a higher IDR than for pure crystalline carvedilol. The highest IDR was observed with aspartic acid. In the case of co-amorphous mixtures involving only weak molecular interactions, it is suggested to select the amino acid and the molar ratio providing the highest T_g. Dry storage conditions are desirable to extend the physical stability of co-amorphous mixtures. Carvedilol-aspartic acid 1:1 molar ratio freeze dried exhibited a significantly better physical stability under room conditions than its ball-milled counterpart, underlining the importance of the amorphisation method. Carvedilol-aspartic acid 1:1 formulated into a tablet showed a systematic recrystallisation of carvedilol in solution.

Finally, a decision tree was elaborated to guide the development of amino acid-based co-amorphous systems.

Zusammenfassung

Eiman Atiek, *Formulierung von schwer wasserlöslichen Arzneistoffen in koamorphen Arzneistoffsystemen mit Aminosäuren: Herausforderungen und Möglichkeiten*, 2018.

Schwer wasserlösliche Arzneistoffe bleiben eine Herausforderung bei der Entwicklung von Arzneimitteln. Viele Strategien existieren, um die Löslichkeit eines Arzneistoffes zu erhöhen; eine Strategie ist die Koamorphisierung des Arzneistoffes mit einer Aminosäure. Die Verwendung von Arzneistoffen zusammen mit Aminosäuren für die Amorphisierung kann den Erfolg der Amorphisierung erhöhen und die physikalische Stabilität der koamorphen Mischung verbessern.
Carvedilol wurde für dieses Forschungsprojekt als repräsentativer Arzneistoffkandidat aus der BCS-Klasse II verwendet. Zunächst wurde die Amorphisierungsfähigkeit von Carvedilol mit verschiedenen Amorphisierungsmethoden untersucht: die schnelle Abkühlung einer Schmelze (*melt quenching*), Gefriertrocknung (*freeze drying*) und Kugelmahlung (*ball milling*). Darüber hinaus wurden die Vorteile und Grenzen jeder Methode bewertet. Danach wurden die erhaltenen koamorphen Gemische unter Verwendung von verschiedenen analytischen Techniken (XRPD, DSC, NMR, FTIR, usw.) charakterisiert. Schließlich wurde das Auflösungsprofil einer koamorphen Tablettenformulierung bestimmt.
Keine der getesteten Amorphisierungsmethoden kann als eine ideale Methode angesehen werden. Die drei Methoden wurden in Bezug auf die Flexibilität in der Auswahl der Stoffe, die Durchführbarkeit und die physikalische Stabilität der resultierenden amorphen Substanzen bewertet. Die Koamorphisierung durch Kugelmahlung war mit neutralen Aminosäuren erfolgreich, allerdings zeigten die Mischungen nur schwache intermolekulare Wechselwirkungen. Die Amorphisierung durch Gefriertrocknung war mit Asparaginsäure erfolgreich und bildete ein koamorphes Salz mit Carvedilol. Die Analyse des thermischen Verhaltens zeigte, dass die gefriergetrockneten Mischungen physikalisch stabiler gegen Erwärmung sind als kugelgemahlene Mischungen, die eine systematische Rekristallisation oberhalb der Glasübergangstemperatur (T_g) zeigten. Die T_g lieferte wertvolle Informationen für die Auswahl der Aminosäuren und das bevorzugte Stoffmengenverhältnis mit Carvedilol. Die Hygroskopizität des kristallinen Ausgangsmaterial führte zu einenem schlechteren Erfolg bei der Amorphisierung. Koamorphisierung von Carvedilol mit den ausgewählten Aminosäuren verringerte die scheinbare Wasserlöslichkeit von Carvedilol. Die intrinsische Auflösungsgeschwindigkeit (IDR) war in der koamorphen Form jedoch systematisch höher als bei der entsprechende kristalline physikalische Mischung. Die physikalische Mischung selber weist eine höhere IDR als reines kristallines Carvedilol. Die höchste IDR wurde mit Asparaginsäure beobachtet. Ein Ergebnis dieser Arbeit stellt dar, dass im Fall von koamorphen Systemen mit nur schwachen molekularen Wechselwirkungen, die Aminosäure und das Molverhältnis gewählt werden sollte, die die höchste T_g aufweisen. Trockene Lagerbedingungen sind wünschenswert, um die physikalische

Stabilität von koamorphen Mischungen zu verlängern. Gefriergetrocknete Carvedilol-Asparaginsäure in Molverhältnis 1:1 zeigte eine deutlich bessere physikalische Stabilität unter Raumbedingungen als sein kugelgemahlenes Äquivalent. Dieses Ergebnis unterstreicht die Bedeuteung der Amorphisierungsmethode. Tablettenformulierungen aus koamorphen Carvedilol-Aminosäure 1:1-system zeigten systematisch Rekristallisation von Carvedilol in der Lösung.
Schließlich wurde ein Entscheidungsbaum erstellt, um die Entwicklung von Arzneistoff-Aminosäure-Systemen zu begleiten.

1. Introduction

The poor aqueous solubility of active pharmaceutical ingredients (API) has become a great challenge in the last years [1, 2]. The proportion of marketed poorly water soluble drugs has increased [2–6]. Poor solubility may affect the bioavailability by reducing the absorption extent of the drug orally administered [4]. Indeed, the dissolution rate of the API in gastrointestinal fluids can be the limiting factor for the absorption of the drug [4, 5]. Over the last decades the research efforts on the enhancement of drug solubility intensified in order to overcome this challenge.
A classification of the APIs was established by Amidon *et al.* [4] and is used by the FDA as a guidance for industry [7] on the basis of two criteria: the aqueous solubility and the intestinal membrane permeability of the drug. This *Biopharmaceutics Classification System (BCS)*, is divided in four classes:

Class I	High solubility and high permeability
Class II	Low solubility and high permeability
Class III	High solubility and low permeability
Class IV	Low solubility and low permeability

The permeability and the solubility are considered to be the two main parameters controlling drug absorption [4]. Class I of this classification represents the ideal case for a drug in terms of absorption and bioavailability. Class II together with class IV are composed of the largest number of approved drugs [3, 8, 9]. Class II is subjected to intensive research in terms of solubility enhancement through dedicated study programs focusing on representative Class II drugs [10,11]. Class IV comprises a lower number of APIs intended for use in immediate release (IR) oral drug formulations.
The insolubility of a drug is mainly due to two intrinsic factors of the molecule. Either it exhibits a high lipophilic character or the intermolecular forces within the crystal lattice of the molecular arrangement are too strong to permit a rapid dissolution, the Gibbs free energy of the system is largely negative ($\Delta G \ll 0$) [12, 13]. To overcome these two issues a wide variety of strategies are currently explored. Chemical strategies or formulation strategies can be used in order to enhance the apparent solubility or dissolution rate of highly lipophilic compounds [14]. Chemical strategies include, for example, the derivatisation of the drug (with the intent to alter its acido-basic properties) [15] and the synthesis of prodrugs [16]. Formulation strategies include, for example, the use of co-solvents [17,18], micelles technologies [19] and the complexation of the drug [20, 21].
To reduce intermolecular forces within the crystal lattice other strategies can be used, for example, salt formation [22–26], solvate formation [26–28] or cocrystallisation with another molecule, which besides might have a therapeutic relevance [24, 29–34]. In addition, other strategies involving metastable forms of the API, such as polymorphs [26, 35] and amorphous solid forms [36, 37] can be used for formulations.
One of the possible formulation strategies is the amorphisation of the drug with a co-former as physical stabiliser. When the drug and the co-former both exhibit a low

molecular weight the formed amorphous mixture is called a co-amorphous system.
The present thesis deals with a metastable form of drugs, the amorphous state, and more specifically with co-amorphous systems involving amino acids. In this research work, carvedilol was selected as a basic drug belonging to class II of the BCS [9]. Carvedilol was selected because of its low aqueous solubility and its basic properties (pK_a=7.8). This latter characteristic is important due to the low number of basic drugs investigated historically as co-amorphous systems involving amino acids.

1.1. Co-amorphous drug systems

Co-amorphous drug systems are by definition mixtures in a metastable state. The co-amorphous systems are homogeneous and monophasic amorphous mixtures involving two or more low molecular weight molecules including at least one drug [38]. They are classified as a particular case of amorphous solid dispersions, because they involve only low molecular weight molecules, whereas an amorphous solid dispersion may also involve polymers [38–40].
The co-amorphous mixtures potentially enhance the solubility and the dissolution rate of the drug while strengthening the stability of the amorphous state by involving the co-former as a stabilising agent (see below) [41, 42]. The solubility and/or dissolution are potentially enhanced due to the high energetic solid state. In theory, the solid state forms of higher energy structure should exhibit increased solubility and dissolution properties [41,43]. Some experiments showed, in practice, that single amorphous drugs may have a significantly enhanced dissolution profile [41, 44]. Despite an increased dissolution rate or solubility, in many cases the amorphous material recrystallised upon exposure to solvents since the supersaturated state of the solution containing the drug is difficult to maintain [45].
Since the amorphous state is a highly energetic state, it is usually considered less stable than the lower energy crystalline form. Many research projects aim to stabilise the amorphous state of the drug by various techniques such as formulating it in a glass solid dispersion with a polymer, co-amorphisation with an other therapeutically-relevant drug, or co-amorphisation with an excipient of low molecular weight. The role of the co-former (polymer or low molecular weight molecule) is to prevent the drug from recrystallisation by creating a physical barrier between drug molecules. Indeed, when the drug is successfully formulated in a homogeneous amorphous mixture, the intermolecular interactions between the drug and the co-former prevent the drug from recrystallisation and in addition generally involve weaker intermolecular interactions than between the drug molecules in the most stable crystalline form. This permits an enhanced solubility or dissolution rate. The use of an amorphous co-former aims to stabilise the amorphous state of the drug by improving specific physical characteristics such as increasing the glass transition temperature and raising the supersaturated level of the API upon dissolution [46].
When comparing the use of polymers with low molecular weight molecules employed as co-formers, each approach has distinct advantages and disadvantages (summarised in table 1.1). Polymeric amorphous solid dispersions (PASD) are better known and already a well-established method of formulation aiming to enhance the solubility or dissolution rate of an API. Marketed oral formulations already exist, such as amorphous ritonavir formulated with PVP/VA (Norvir®, Abbott) [47]. The interaction

mechanisms are partially known and remain under investigation. However, polymers exhibit some limitations in their usage. Many low molecular weight drugs have a low solubility in the selected polymer, which requires the use of a larger amount of polymer to reach the needed miscibility. This leads to a larger volume of the final formulation. Moreover, polymers are generally very hygroscopic, which is in general a non-desirable property in terms of chemical and physical stability. Indeed, the water attracted into the mixture may cause a recrystallisation of the drug by increasing the molecular mobility and decreasing the T_g [46,48]. On the other hand, PASD showed the ability of the polymer to stabilise the supersaturation of the drug upon dissolution and delays its recrystallisation. This point is of great importance and constitutes one of the main objectives of amorphous drug-based formulations. Co-amorphous systems, in turn, can reach a higher level of miscibility via more specific intermolecular interactions, herewith limiting the necessary amount of co-former [46]. However, co-amorphous mixtures remain little known and many involved mechanisms (co-amorphisation mechanisms, choice of the co-former, long-term stability, hygroscopicity, etc.) require further investigations.

Table 1.1. Advantages and disadvantages of polymeric amorphous solid dispersions (PASD) and co-amorphous systems.

	Advantages	**Disadvantages**
PASD	• longer stability of the amorphous state • polymer helps maintaining the supersaturation • better knowledge and more experiences	• poor solubility of the drug in the polymer • larger bulk volume of the final formulation • high hygroscopicity of the polymers • phase transformation/separation
Co-amorphous systems	• more specific intermolecular interactions • mixing at molecular level	• probable shorter supersaturation • mechanisms not well understood yet

The fist occurrence of the term "co-amorphous system" appears in 2009 in a publication by Chieng *et al.* [49]. However, the concept of using two melt-quenched small molecules was already evoked in 1965 by Glodberg *et al.* under the name "glass solutions", when describing sulfathiazole-urea and chloramphenicol-urea mixtures [50,51]. This concept of combining two low molecular weight molecules into an amorphous system was further investigated with other high aqueous-soluble small molecules as co-former such as citric acid [52] or saccharin [53]. The first co-amorphous system composed of two drugs, cimethidine and indomethacine, was prepared and described by Yamamura *et al.* [54]. Such systems gained again interest in the last decades probably due to the increasing number of poorly water-soluble drugs in development.
Löbmann *et al.* first proposed to use amino acids as excipient co-formers for co-amorphisation [55,56]. The main motivations were based on the amino acids being part of the binding site of the pharmacological target. If the drug and the amino acid were able to bind at the target site, then the same molecular interactions would also allow a co-amorphous mixture containing both substances to be formulated [55]. The other rationale was that amino acids are regarded as toxicologically safe.
Many drugs have been already formulated in co-amorphous systems using amino acids on a laboratory scale, such as carbamazepine [55], indomethacine [55, 57, 58], naproxen [55], glibenclamide [59], simvastatine [59], valsartan [60], carvedilol [61], mebendazole [61] and furosemide [57,61].
The intermolecular interactions have been investigated in numerous research projects [10, 55] and it was deducted, first of all, that the presence of the amino acid in the

binding domain of the pharmacological target is not a necessity for the co-amorphisation [55]. This is consistent with the fact that the three-dimensional interactions between the binding domain and the drug do not necessarily represent the drug-amino acid interactions existing in a co-amorphous mixture. Secondly, the intermolecular interactions between the drug and the amino acid could be divided into weak (hydrogen bonding, π-π interactions, etc.) and strong interactions, which include salt formation. These two types of interactions lead to different physicochemical properties of the co-amorphous systems. For instance, co-amorphous salts exhibited an enhanced physical stability [62]. The co-amorphous salt formation is nowadays under investigation and a first observation was that salt formation is independent from the chemical structure of the co-former [63].
The selection of the molar ratio was also investigated by Jensen *et al.* for indomethacine and furosemide co-amorphised with three selected amino acids [64]. A screening method was studied to guide the selection of the amino acids which are expected to form a completely amorphous mixture [61]. This method consists in ball milling the drug with various naturally occurring amino acids and assuming that a decrease of crystallinity by 90 % after 15 min of milling – as detected by XRPD – implies good probability of complete amorphisation.
Finally, Lenz *et al.* formulated spray-dried co-amorphous indomethacine-arginine into a tablet and showed that the processing of this co-amorphous salt did not alter the physical stability of the system [58, 65].
Many aspects of amino acid co-amorphous-based systems remain to be explored. The amorphisation methods are still poorly investigated and their influence on the final substance is unknown. The factors enabling co-amorphous salt formation are also to be investigated. Many physicochemical characteristics of the co-amorphous systems need to be investigated with regard to potential downstream processing. The long-term stability of theses systems has not yet been studied and the elaboration of guidelines for the selection of the "right" amino acid is an exciting objective, knowing that amino acids constitute an interesting panel of safe potential co-formers with a variety of physiochemical properties (pK_a, solubility, etc.).

1.2. Amorphisation methods

The co-amorphisation of a drug can be performed via various methods. They can be grouped in three distinct categories based on the amorphisation mechanism. The first group comprises thermal methods such as melt quenching (or quench cooling) and hot melt extrusion. These two methods involve a phase transformation of the solid crystalline material into a melt and followed by a cooling phase. Both methods can be unsuitable for thermosensitive substances such as amino acids depending on the working temperature. Moreover, hot melt extrusion implies necessarily the use of polymers in addition to the co-amorphous substance for the process to be feasible. Lenz *et al.* described an example of a successful hot melt extrusion process involving indomethacine-arginine combined with a polymer. However, arginine was previously dissolved in a solvent to avoid degradation from melting at high temperature [65].
The second category comprises mechanical methods, such as ball milling and cryomilling. Ball milling is the most commonly used method for co-amorphisation of drugs with amino acids [55, 57, 66]. Both methods have the advantage of requiring

working temperatures low enough to avoid thermal degradation of the substances. Both methods also allow the amorphisation to be performed in dry conditions or with the use of a small amount of solvent [67].
The third category comprises methods involving solvent evaporation such as rotary evaporation, spray drying and freeze drying. These methods imply the initial dissolution of the substances to be amorphised followed by the evaporation of the solvent. By controlling the kinetics of evaporation, the drug molecules remain in a disordered configuration and can be obtained in the amorphous state. The main challenge of these methods when attempting the co-amorphisation of a drug with an amino acid is to find an adequate solvent capable of dissolving both substances in a reasonable volume. For this purpose a mixture of solvents could be used. Co-amorphisation by spray drying was already tested with the indomethacine-argine system [62]. Other solvent evaporation methods were tested with drug-drug co-amorphous systems [54] and methods like amorphisation by dehydration [68] led researchers to believe in the existence of "polyamorphism" meaning the presence of different amorphous forms [69]. This is likely to have a pharmaceutical relevance particularly regarding the different physical properties of the amorphous forms obtained from the same substance by different amorphisation techniques [70].
Methods involving solvent evaporation, spray drying and melt extrusion proved their ability to be adapted for large-scale production [46].

1.3. Characterisation techniques for amorphous solids

There are many characterisation techniques for amorphous solids. The most commonly used methods aim, in the case of co-amorphous systems, to examine specific characteristics such as thermal behaviour, the amorphisation level and the potential intermolecular interactions.
X-ray powder diffractometry (XRPD) is mostly used to determine the amorphisation level of co-amorphous systems. Indeed, amorphous solids characterised through standard laboratory XRPD analysis exhibit no reflection peaks in their XRPD pattern and show instead a typical halo representative of amorphous solids. This is the main application of this technique for co-amorphous systems. However, XRPD was also used in a quantitative manner to monitor the amorphisation progress and develop a screening method towards the choice of amino acids as a co-former [61].
Spectroscopic techniques are used for the assessment of potential intermolecular interactions in the amorphous state. The most commonly used techniques are solid state nuclear magnetic resonance (ssNMR), Raman spectroscopy, near infrared spectroscopy (NIR), infrared spectroscopy (IR) and terahertz pulsed spectroscopy [10]. Fourier Transform Infrared Spectroscopy (FTIR) is one of the easiest and most used techniques for the analysis of intermolecular interactions [71].
Differential scanning calorimetry (DSC) is a thermal technique typically used for the characterisation of the thermal behaviour of amorphous solids. It allows a qualitative analysis of the amorphous properties of a substance such as the detection of potentially polymorphic behaviour. A quantitative analysis is also in some cases possible. One of the most important properties measurable by DSC in amorphous solids is the glass transition temperature. It represents the temperature at which an amor-

phous solid converts from a glassy solid state into a rubbery state upon heating. Both are amorphous phases but exhibit different physical properties, particularly in terms of viscosity and internal thermal energy. The glass transition temperature is of high importance for the stabilisation strategies of solid amorphous systems. Beside those described above, there are multiple other techniques for the characterisation of amorphous solids. Only the most important techniques for this research project are mentioned.

1.4. Predicting the T_g: The Gordon-Taylor equation

The T_g is an important parameter for the characterisation of amorphous solids. Indeed, below and above the T_g the physical properties do change significantly. When two or more substances are mixed in an amorphous state, the T_g varies as a function of the composition. The *in silico* prediction of the T_g is hence advantageous.
The Gordon-Taylor equation is the most commonly used model equation for the prediction of the T_g of the co-amorphous system. This equation is nowadays considered the most adequate for low molecular weight-based amorphous mixtures, whereas the Fox-Flory equation remains more accurate for systems using large molecular weight polymers [72].
Gordon and Taylor developed this equation in 1952. It predicts the T_g of the final co-amorphous mixture as a function of the single T_g of both the individual substances and the mass proportion of each component (equation 1.1) [73].

$$T_{g12} = \frac{w_1\, T_{g1} + K\, w_2\, T_{g2}}{w_1 + K\, w_2} \tag{1.1}$$

where

T_{g12}	glass transition temperature of the co-amorphous mixture
T_{g1}, T_{g2}	glass transition temperatures of the single substances composing the mixture
w_1, w_2	mass proportions of the single components
K	a constant defined by equation 1.2.

This equation includes the parameter K, which is a fitting parameter. Simha and Boyer, in 1962, proposed equation 1.2, which takes into account the density of the mixed components [74].

$$K = \frac{T_{g1}\, \rho_1}{T_{g2}\, \rho_2} \tag{1.2}$$

where

ρ_1, ρ_2	true densities of the single components in their amorphous form.

However, this fitting parameter was originally developed for polymeric systems. In the case of small molecules, this fitting parameter K as defined by equation 1.2 remains valid and describes ideal co-amorphous systems. As the amorphisation of single compounds such as amino acids can be challenging, the density of each component is difficult to measure. Therefore, as an approximation of the density of each single compound, the density of the most stable or most common crystalline form is used.
Recently, Jensen *et al.* investigated the use of the Gordon-Taylor equation for the determination of the molar ratio between the drug and the amino acid exhibiting the strongest intermolecular interactions [64]. The T_gs at different ratios for a given

co-amorphous drug-amino acid system were calculated with the Gordon-Taylor equation and compared with the measured values. The Gordon-Taylor equation assumes an ideal behaviour of the mixtures and the potential intermolecular interactions, which can be strong (from amorphous salt formation, for example), are not taken into account. Jensen *et al.* assumed that the co-amorphised mixture exhibiting the measured T_g most dissimilar from the calculated T_g exhibits the strongest intermolecular interactions and hence should be the most physically stable ratio.

1.5. Solubility and dissolution

Aqueous solubility is one of the factors permitting the classification of the drugs in the BCS, and is considered one of the possible limiting parameters for a low bioavailability [4].
The solubility of a solid substance is usually defined at a precise temperature and in a defined medium. The values of solubility represent generally the solubility of the substance in a saturated solution at equilibrium. A solubility which could be measured from a non-equilibrated state is designated as apparent solubility. There are various methods to measure the solubility, one of the most common being the shake-flask method, where a flask containing a saturation proportion of solid in a fluid is shaken at a constant temperature and for a defined period of time. A sample of the fluid is removed and the concentration of the previously filtered solute can be determined by various analytical techniques (UV, titration etc.).
The dissolution rate of a substance can also be improved by co-amorphisation. The dissolution profile of a substance can be determined either for the powder or from a formulation involving additional excipients. The dissolution profile is also dependent on the contact surface between the solid and the solvent. For an easier comparison of the dissolution rates of pure substances, the intrinsic dissolution rate is often used. Intrinsic dissolution experiments allow the contact surface between the sample and the medium to be constant, herewith eliminating this variable.

1.6. Aims of the thesis

In this thesis, carvedilol will be used as a representative drug candidate from BCS class II.
Firstly, the amorphisation ability of carvedilol either alone or in combination with an amino acid will be assessed. One basic amino acid, L-arginine, two neutral amino acids, L-phenylanine and L-tryptophan, and two acidic amino acids, L-glutamic acid and L-aspartic acid are selected for the co-amorphisation at three molar ratios (1:3, 1:1 and 3:1). Three amorphisation methods will be tested, melt quenching (MQ), freeze drying (FD) and ball milling (BM). In addition, the advantages and limitations of each method will be evaluated.

Secondly, the co-amorphous mixtures obtained will be characterised using a range of analytical techniques (XRPD, DSC, NMR, FTIR, etc.).
The success of amorphisation will be assessed by X-ray powder diffractomety (XRPD), the thermal behaviour of the co-amorphous mixtures will be analysed by differential

scanning calorimetry (DSC), and the intermolecular interactions between carvedilol and the amino acid will be determined by FTIR and NMR.
Moreover, the apparent solubility of the co-amorphous substances will be determined in an acidic aqueous solvent at 37 °C and the intrinsic dissolution rate likewise.
A stability study will be performed to determine the influencing factors towards an enhanced physical stability of the co-amorphous systems with carvedilol. Factors such as the selected amino acid, the molar ratio between carvedilol and the amino acid and the humidity level of the surrounding environment will be investigated.
Based on these results, a coherent summary will be presented in order to assess the key factors for successful co-amorphous systems containing a BCS class II drug, to define the remaining challenges and summarise the acquired knowledge.

Finally, a tablet formulation containing a selected carvedilol-based co-amorphous system will be developed and its dissolution profile will be established and discussed.

2. Results and Discussion

2.1. Amorphisation

There are many possible amorphisation methods. Regarding the choice of the method, it is important to take several parameters into account. First of all, it is important to consider the physiochemical properties of the substances to be amorphised, their thermostability, their thermal transitions (melting point, glass transition, etc.) and their chemical stability. Secondly, the aimed downstream process of the obtained amorphous substance (for example tabletting) is also of great importance in the choice of the amorphisation method. This, due to the physical properties of the obtained amorphous substance, more or less suited for the processing.

Different methods were used for obtaining either pure amorphous substances (either carvedilol or amino acids) or co-amorphous mixtures. These were always binary mixtures and composed of carvedilol (Car) and an amino acid. This section focuses primarily on the process of amorphisation.

2.1.1. Amorphisation of pure substances

The ability of pure crystalline substances to shift into the amorphous state was assessed as well as their stability in this state. Amorphisation of starting materials allowed eventually to identify their glass transition temperature.
Carvedilol is a thermostable substance (melting is a reversible change) and was amorphised by two different methods: (1) by melt quenching (MQ) (section 3.2.2), and (2) by ball milling (BM) (section 3.2.1).
The amino acids were amorphised by BM only. Their thermostability is insufficient, showing decomposition at 150 °C to allow MQ to be applied.

2.1.2. Amorphisation of drug-amino acid mixtures

For obtaining co-amorphous mixtures with carvedilol as the drug and an amino acid as a co-former, ball milling was used. In one instance (Carvedilol-Aspartic acid pair at 1:1 molar ratio) freeze drying (FD) (section 3.2.3) was also used.

2.1.3. Properties of obtained powders

The powders obtained from the different amorphisation methods show distinct properties at a macroscopic level. As represented in figure 2.1, it is possible to distinguish them with respect to particle shape, particle size and also density. The ball-milled and melt-quenched powders are visually more dense than the freeze-dried powder.

Figure 2.1. Freeze-dried Car-Asp at 1:1 molar ratio (a), melt-quenched carvedilol (b) and ball-milled Car-Asp at 1:1 molar ratio (c).

Freeze-dried powders

The freeze drying process was performed with water as solvent in line with the equipment specifications, which do not allow the use of organic solvents. As carvedilol is poorly water soluble, a large amount of water was needed to dissolve it (approx. 500 ml of Water for 25 mg of Car-Asp 1:1 physical mixture). After sublimation of the water an XRPD measurement was systematically performed to assess the level of amorphisation of the mixture. Most of the time the XRPD patterns showed residual crystallinity. Therefore, the obtained FD powder was dissolved again in 500 ml of water and freeze dried a second time. Eventually, the powder obtained was amorphous. The use of this large amount of water for the FD process gave rise to a powder with a visibly low density, heterogeneous particle size (figure 2.1.a) and electrostatically charged, such that the downstream process became more complex. However, this type of powder turned out to have better dissolution properties in liquids than other types of amorphised powder obtained by MQ or BM.

Melt quenched powders

Melt quenching is the easiest way to get the substances amorphous. However, as previously mentioned, this process is adequate only if the substance shows a reversible transition between the solid and the liquid state. The substance obtained after melting and then quenching is, in the case of carvedilol, a glassy and transparent material. As the latter is not a powder, it is necessary to crush it although the crushing process could be a cause of recrystallisation. The chosen method was to gently crush carvedilol in a mortar with a pestle until a fine and homogeneous powder was obtained (see figure 2.1.b). An XRPD measurement was systematically performed immediately after crushing to ensure that the mixture was still completely amorphous. Melt quenching has the advantage of being easy to perform and also offers the possibility to control the particle size of the final powder.

Ball-milled powders

Ball milling was the main amorphisation method used in this project. It represents a good compromise in terms of process complexity while offering flexibility regarding the range of substances to be amorphised. Ball milling is a suitable amorphisation method

for thermosensitive substances such as amino acids (AA). A planetary ball mill placed in a cold room at 5 °C was used. Cooling is necessary for two reasons: (1) the substance needs to be milled below its glass transition temperature (T_g) [43] and (2) the milling itself generates heat, which increases the temperature in the milling jar. In terms of milling time (10 −15 h), a comparison was made with experiments from Kasten *et al.* [61], where a vibration ball mill was used to mill mixtures containing carvedilol. The milling time was shorter in their experiments since they used a mill with a higher energy input. The main disadvantage of the planetary ball mill is the aspect of the obtained powder. After 10 −15 h of milling, the carvedilol or carvedilol-AA mixtures present some very fine particles and also unexpectedly coarse particles (figure 2.1.c). Immediately after milling, the powder forms a large fragment sticking to the bottom of the milling jar (figure 2.2). It was always necessary to crush the fragment manually in order to detach the powder. This led to a heterogeneous particle size and, therefore, poor flowability. Another consequence of this sticking phenomenon was the impossibility to amorphise completely a substance or a mixture more than 1 g at a time in 25 ml milling jar.

Figure 2.2. Milling jar containing ball-milled Car-Asp at 1:1 molar ratio.

2.1.4. Summary: advantages and disadvantages of the amorphisation methods

The table 2.1 summarise the advantages and disadvantages of methods used for the amorphisation.

Table 2.1. Advantages and disadvantages of freeze drying (FD), melt quenching (MQ) and ball milling (BM) for the amorphisation of carvedilol alone or in mixtures with amino acids

Method	Advantages	Disadvantages
FD	• rapid re-dissolution • better stability of the amorphous state (will be discussed in paragraph 2.2.7)	• complex process • "cotton wool" texture (non-particulate) ⇒ poor flowability • electrostatically charged
MQ	• easy process • particle size can be controlled through crushing ⇒ good flowability	• not suitable for thermosensitive substances (such as AA)
BM	• suitable for all kind of substances	• requires a cooled equipment long milling time (depending on the substance and the type of mill) • uncontrolled particle size ⇒ poor flowability • lower stability of the amorphous state (see section 2.2.7)

It is difficult to clearly define one method as the best method for amorphisation. As explained throughout this section, the choice of the method depends on the final use of the powder as well as the properties of the substances to be amorphised.

2.2. Characterisation

Co-amorphous mixtures were characterised with a range of analytical methods. Firstly, XRPD measurements were performed to assess the success of the amorphisation process. Secondly, DSC measurements allowed the characterisation of the thermal behaviour of the co-amorphous mixtures and the determination of the glass transition temperature. Thirdly, FTIR and NMR measurements were performed to assess the nature of the interactions between carvedilol and the amino acids in the amorphous state. Then, the solubility and the intrinsic dissolution of co-amorphous mixtures were measured in order to evaluate the benefit of co-amorphous mixtures in terms of solubility enhancement. Finally, a stability study was conducted to determine the influence of the ambient humidity on the physical stability of co-amorphous mixtures.

2.2.1. Evaluation of the success of amorphisation

The success of amorphisation was assessed by X-ray powder diffractometry (XRPD)(the method is described in section 3.2.6). The absence of peaks on the XRPD patterns indicates the total amorphisation of the ball-milled substances. Instead, a typical halo for amorphous structure is visible.
Factors that may influence the success of amorphisation were studied. The nature of the amino acid used as a co-former for the amorphisation was studied. Accordingly, acidic, basic and neutral amino acids were used for the amorphisation. The ratio between carvedilol and the amino acid was also studied. Finally, a comparison between ball-milled and freeze-dried mixtures was made through XRPD measurements.

Acidic, basic and neutral amino acid for the co-amorphisation of carvedilol

Ball-milled mixtures composed of carvedilol and one amino acid were measured by XRPD. Accordingly, a basic amino acid, L-arginine (Arg), two neutral amino acids, L-phenylalanine (Phe) and L-tryptophan (Trp) and finally two acidic amino acids, L-glutamic acid (Glu) and L-aspartic acid (Asp) were used.

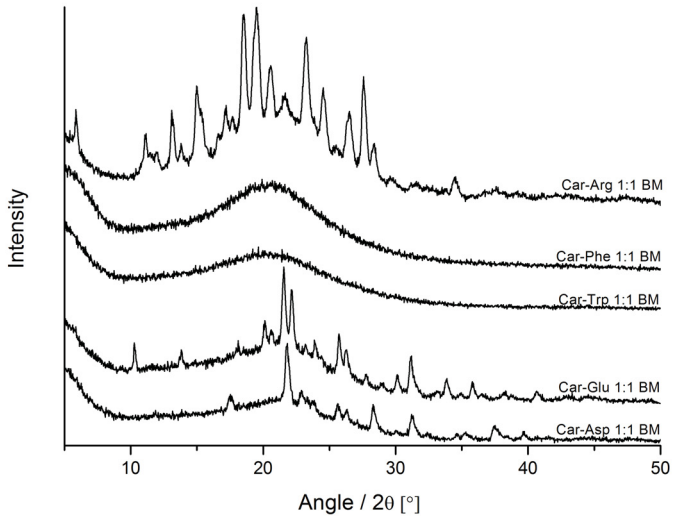


Figure 2.3. XRPD diffractograms of ball-milled Car-Arg, Car-Phe, Car-Trp, Car-Glu and Car-Asp at 1:1 molar ratio.

Figure 2.3 represents the XRPD patterns of carvedilol ball milled with the different amino acids at 1:1 molar ratio. It is observed that only co-amorphisation with Phe and Trp was fully successful. Neither acidic nor basic amino acids were good co-formers. Co-amorphisation with Arg showed reflections corresponding to residual crystalline carvedilol and Arg (diffractograms of reference for single crystalline components are presented in appendix A.1). In the case of co-amorphisation with acidic amino acids, reflections corresponding to Glu and Asp were respectively observed.
It appears that charged amino acids are not good co-formers for co-amorphisation with carvedilol. However, XRPD alone as a characterisation method is unable to highlight the best co-former. An ideal co-amorphisation occurs when the drug and the amino acid form a homogeneous mixture without long range order [38]. However, XRPD cannot differentiate whether it is an homogeneous mixture or not.

Co-amorphisation of carvedilol with amino acids at different ratios

Carvedilol was ball milled with the different amino acids at ratios of 3:1 and 1:3 in addition to the 1:1 molar ratio. XRPD measurements were performed to determine whether the relative proportion of carvedilol with regard to the amino acid has an influence on the success of amorphisation.

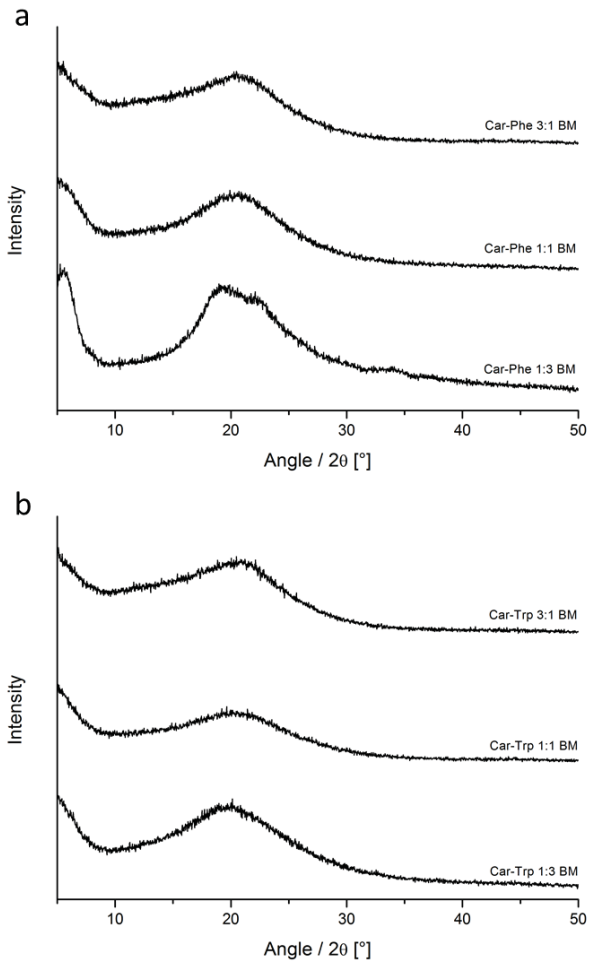


Figure 2.4. XRPD patterns of ball-milled Car-Phe (a) and Car-Trp (b) at 3:1, 1:1 and 1:3 molar ratios.

Neutral amino acids Figure 2.4 shows the XRPD patterns for ball-milled carvedilol with either Phe or Trp at 3:1, 1:1 or 1:3 molar ratios. In every case the amorphisation was successful. Therefore, it is not possible to determine which ratio is the best exclusively on the basis of XRPD measurements. However, it is possible to observe a difference in the shape of the halo (typical for the amorphous structure) depending on the ratios of each carvedilol amino acid pair. These shapes depend generally on the equipment used for the measurements and also on the amorphous structure itself. In this case, all the measurements were performed with the same equipment. Other analytical methods, such as DSC, are required to distinguish the differences between the mixtures.

Acidic and basic amino acids Figure 2.5 shows that co-amorphisation with either Arg, Glu or Asp is unsuccessful regardless of the ratio at which the mixture is ball milled. Figure 2.5.a, shows residual crystallinity of carvedilol and Arg. When carvedilol is present in the mixture in higher proportion the carvedilol XRPD pattern is more intense than that of Arg.
Figure 2.5.b represents the different ratios of carvedilol Glu mixtures. In all three patterns only reflections corresponding to Glu are visible. In a similar way, on figure 2.5.c, only the reflections of Asp are visible.

There is certainly a difference between the basic amino acid, Arg and the acidic amino acids Glu and Asp regarding the amorphisation by ball milling. In the first case, traces of crystalline carvedilol are still visible. This means that Arg has a negative influence on the ability of carvedilol to be amorphised. Oppositely, in figure 2.5.b (Car-Glu), and 2.5.c (Car-Asp), no traces of crystalline carvedilol could be observed on the diffractograms.

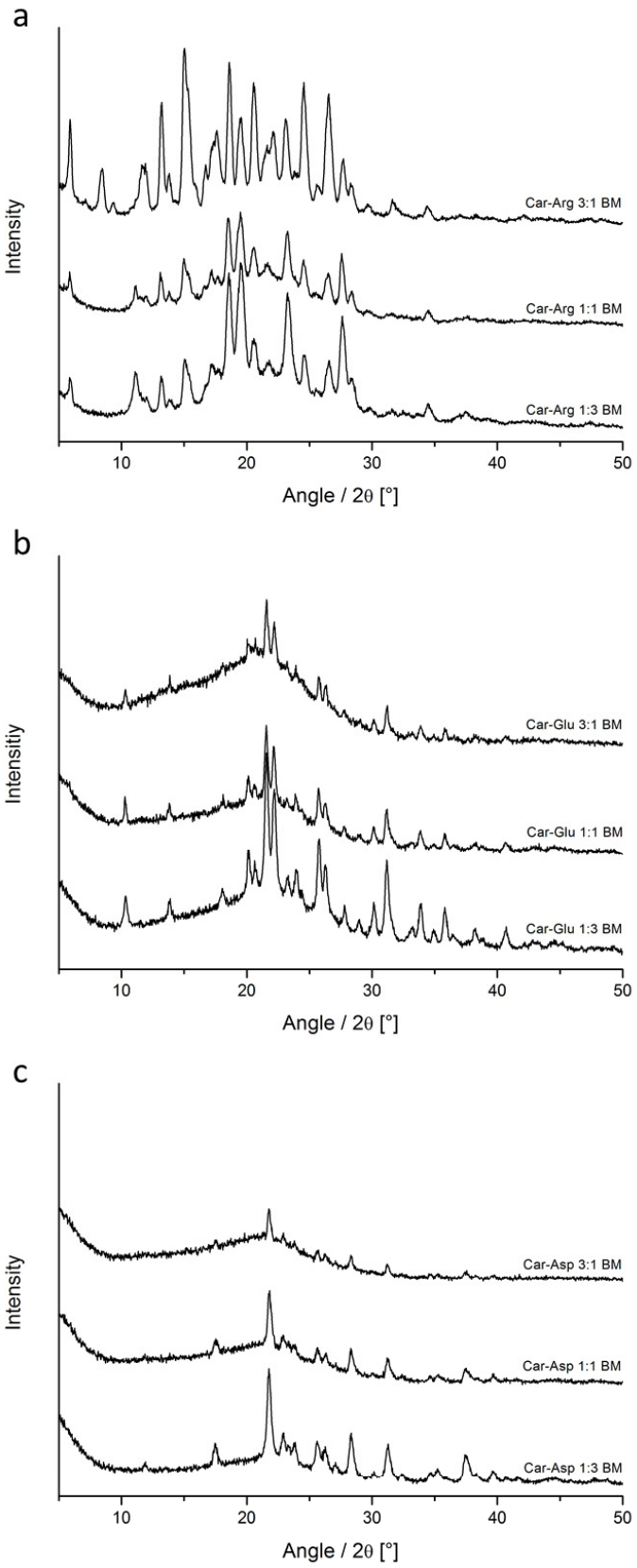


Figure 2.5. XRPD patterns of ball-milled Car-Arg (a), Car-Glu (b) and Car-Asp (c) at 3:1, 1:1 and 1:3 molar ratios.

Comparison between ball-milled and freeze-dried carvedilol-aspartic acid mixtures

XRPD measurements were performed on ball-milled and freeze-dried Car-Asp mixtures in order to compare the success of amorphisation with both methods of amorphisation. As described in section 2.1, Car-Asp 1:1 mixtures were ball milled and freeze dried twice in succession. After each freeze drying cycle, an XRPD measurement was performed. As previously mentioned, ball-milled Car-Asp mixture at 1:1 molar ratio was not completely amorphous (figure 2.6). By contrast, freeze-dried Car-Asp 1:1 after the first freeze drying cycle (figure 2.6, FD - 1) was almost amorphous. Two peaks at 5.70° and 9.64° correspond to a residual crystallinity of carvedilol. However, after the second freeze drying process the mixture was totally amorphous (figure 2.6, FD - 2).
This result shows that the process of amorphisation can affect the success of amorphisation. In the case of the Car-Asp 1:1 mixture freeze drying appears to be a more effective method of amorphisation than ball milling.

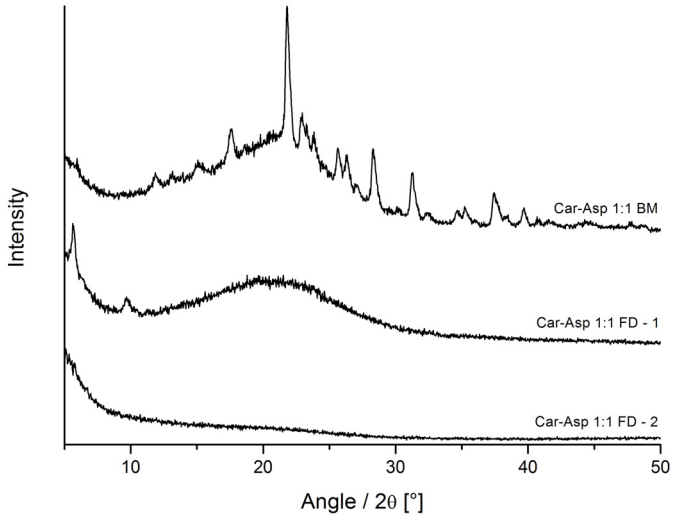


Figure 2.6. XRPD patterns of ball-milled (BM), and freeze-dried (FD) Car-Asp 1:1 molar ratio. FD - 1: Car-Asp 1:1 freeze dried one time, FD - 2: Car-Asp 1:1 freeze dried twice sccssively.

2.2.2. Thermal behaviour of the co-amorphous mixtures

The thermal behaviour of the co-amorphous substances is of great importance. The thermal transitions, such as the glass transition temperature, give indications on the presence of an amorphous form, on the possible crystalline forms and on the physical stability of the substances.
The thermal behaviour of the ball-milled mixtures was measured by differential scanning calorimetry (DSC) (the method is described in section 3.2.5). Theses measurements were performed on the same mixtures already presented in section 2.2.1 for the evaluation of the success of amorphisation by XRPD. The thermal behaviour of carvedilol ball milled with two acidic amino acids, L-glutamic (Glu) acid and

L-aspartic (Asp) acid, two neutral amino acids, L-phenylalanine (Phe) and L-tryptophan (Trp) and one basic amino acid L-arginine (Arg) was characterised using DSC, and this, at different molar ratios. Finally, ball-milled and freeze-dried mixtures of carvedilol and L-aspartic acid at 1:1 molar ratio were also compared.

Thermal behaviour of carvedilol ball milled with the different amino acids

Figure 2.7 represents the thermograms of ball-milled (BM) carvedilol with the different amino acids at 1:1 molar ratios. First, it is possible to observe that each thermogram presents a glass transition, which is identifiable by a step change and is here always the event appearing at the lowest temperature. Then, at least one exothermic event is present and corresponds generally to a recrystallisation [75]. Finally, at least one endothermic event most of the time corresponds to a melting event [75]. As other thermal events could also be detected by DSC, they will be identified and discussed when relevant.

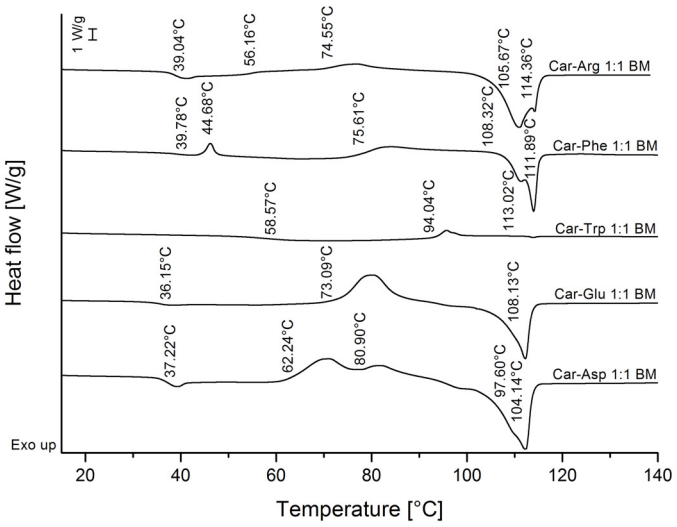


Figure 2.7. DSC thermograms of ball-milled Car-Arg, Car-Phe, Car-Trp, Car-Glu and Car-Asp at 1:1 molar ratio.

The glass transition The glass transition temperature (T_g) is one of the most important thermal events as far as amorphous substances are concerned [43, 70]. It represents the temperature at which the substance turns from a glassy state to a rubbery state. Both states are amorphous phases, however, the rubbery state presents a higher risk of recrystallisation [43]. The temperature of this transition for a co-amorphous mixture depends, among other factors, on the respective T_g of each individual component and their proportion in the mixture. The resulting glass transition of a binary mixture is expected to occur at a temperature situated between the T_gs of both components (see section 1.4) [73, 74]. The T_g of carvedilol was measured immediately after melt quenching crystalline carvedilol and was 35.65 °C. Generally, the glass transition temperatures of investigated amorphous amino acids were higher than that of carvedilol (see appendix A.2 for DSC thermograms of single amorphous

substances).
Each mixture showed a glass transition (figure 2.7), which means that they were all at least partially amorphous. However, the T_g of the different mixtures differs. Most of them have a T_g close to the T_g of carvedilol (35.65 °C, appendix A.2, figure A.2.1). This suggests that they did not form a homogeneous co-amorphous mixture or also that the ratio 1:1 is not the one which enables the best interaction between carvedilol and the amino acid [11]. Only one mixture, Car-Trp 1:1 molar ratio, showed a T_g noticeably higher than for the other mixtures or for amorphous carvedilol. A high T_g is desirable to ensure a better stability for conservation in ambient conditions [43] and also for the potential down stream processes, which usually generate heat. Among the used amino acids for the co-amorphisation at 1:1 molar ratio, Trp seems to present an advantage regarding the resulting T_g.

Recrystallisation behaviour A recrystallisation event on the DSC thermogram of the co-amorphous mixture is generally not desired. An ideal thermal behaviour of a co-amorphous mixture would be to observe only a T_g. However, in most cases a recrystallisation occurs after the glass transition and is inevitable during the experiment. This means that the mixture is also prone to recrystallisation during storage. This is particularly relevant when the T_g is low enough to be close to the ambient temperature, and the recrystallisation occurs shortly after the glass transition as in the present case. Therefore, higher recrystallisation temperatures are advantageous. One particularity of the recrystallisation phenomenon is that it is difficult to predict. Moreover, the recrystallisation temperature could be different between samples of the same composition, even if they were made in the same batch.
It is possible to observe in figure 2.7 that all the mixtures recrystallised upon heating after the glass transition. The recrystallisation temperature itself provides no insight as to which component has recrystallised – carvedilol, the amino acid or both. However, the subsequent melting events observed on the thermogram already provide valuable insight. The Car-Trp 1:1 mixture, once more, shows the best thermal behaviour since it displays the highest recrystallisation temperature at 94.04 °C. In an opposite way, Car-Phe 1:1 recrystallises partially, immediately after the glass transition, such that the estimation of the T_g was difficult. Car-Arg 1:1, Car-Phe 1:1 and Car-Asp 1:1 show two recrystallisations at different temperatures. There could be two interpretations for this: (1) carvedilol and the amino acid recrystallise separately at different temperature and generate two distinct exothermic peaks, or (2) carvedilol recrystallises in two different polymorphs, generating two successive exothermic peaks. In the second case the amino acid could recrystallise either at the same time, or later in the experiment when carvedilol is melting. Alternatively, the amino acid may display no recrystallisation event in case it has not yet occurred by the time the DSC method ends at 140 °C. In the next section it will be shown that the melting is consistent with this interpretation. Therefore the second explanation is the most likely scenario.

Melting events Melting events provide important information on the crystalline form into which amorphous carvedilol recrystallises. There are numerous carvedilol polymorphs and pseudopolymorphs described in the scientific literature, either in patent documentation or journal articles describing their crystallographic structure. Appendix A.3 gives an overview of a selection of patented carvedilol and carvedilol

salt polymorphs and solvatomorphs. The melting temperatures of the polymorphs and pseudopolymorph are indeed different. This information supports the identification of the crystalline modification observed after recrystallisation on the thermograms of figure 2.7.
Figure 2.7 shows that a difference between the mixtures regarding their melting temperature and the number of melting events is observed. The Car-Arg 1:1 ball-milled mixture shows two successive endothermic peaks corresponding to the melting of two crystalline modifications of carvedilol. Indeed, the two previous recrystallisation events lead to this two melting events. The first melting event occurs at around 105 °C and the second at approximately 114 °C, which correspond respectively to the melting point of carvedilol hemihydrate (form III) and carvedilol form II. The latter is the carvedilol form generally marketed and used in this project as the starting substance.
In a similar way, the Car-Phe 1:1 ball-milled mixture shows two melting events which likely correspond to the same two carvedilol modifications. However, the peak intensity (more precisely the peak area) suggests a difference in the proportions of each crystalline form.
Car-Trp 1:1 has a melting event occurring at approximately 113 °C likely corresponding to form II of carvedilol. The low intensity of the melting event indicates a better stability of this mixture upon heating compared to other mixtures. A small intensity (peak area) of the recrystallisation and melting events suggests that the major part of the mixture turned from a glassy amorphous state to a rubbery amorphous state at the T_g, and eventually melted without prior recrystallisation.
The Car-Glu 1:1 ball-milled mixture shows a wide melting point, suggesting that more than one crystalline modification melts within a short temperature range. This melting occurs at 108.13 °C and is consistent with the melting point of carvedilol hemihydrate.
Car-Asp 1:1 presents a double melting peak at approximately 98 °C and 104 °C. Both temperatures are consistent with carvedilol form III and carvedilol form IV (appendix A.3). Carvedilol form III is a hemihydrate and carvedilol form IV is a hydrate [76]. Carvedilol hydrate is described in the literature as non stoichiometric and with a water content of 2 % or more. However, the hydration state is not clearly specified.

The thermal behaviour of carvedilol is different depending on the amino acid used for the coamorphisation. When the Car-Phe 1:1 and the Car-Trp 1:1 mixtures are compared (both being completely amorphous mixtures, see 2.2.1), the glass transition, recrystallisation and melting events occur at different temperatures. This may be interpreted by considering the physicochemical properties of the amino acid, (1) the ability of the amino acid to from interactions with carvedilol, either strong interactions (ionic interactions) or weak interactions (hydrogen bonds, π-π interactions, etc.), (2) the hygroscopicity of the amino acid and its ability to attract water into the mixture, (3) the near range structure of the molecules in the mixture, which might be different depending on the selected amino acid and not measurable with standard laboratory equipment. The first point will be discussed in section 2.2.3, the second point in section 2.2.4 while the third point deserves more investigation and resources to be further developed.

Thermal behaviour of carvedilol ball milled with neutral amino acids at different ratios

The thermal behaviour of ball-milled mixtures was studied for several mixtures at different molar ratios. First, the thermal behaviour of the two successfully amorphised Car-Phe and Car-Trp mixtures is analysed at 3:1, 1:1 and 1:3 molar ratios. The ratio between carvedilol and the amino acid might have an influence on the thermal behaviour of the mixture. Three model scenarios are expected:

1. Carvedilol is in excess in the mixture. Then, the mixture obtained is composed of two phases, one with carvedilol and the amino acid with the ideal ratio for the best intermolecular interactions and an ideal homogeneity in the amorphous state. The second phase is composed of the excess of amorphous carvedilol. The thermal behaviour is then expected to be close to the behaviour of amorphous carvedilol. Alternatively, it is also possible to expect two glass transitions, one for each amorphous phase.
2. The amino acid is in excess in the mixture with expectations reversed to those described in 1. The thermal behaviour is expected to be closer to the behaviour of the amorphous amino acid, and it may similarly show two glass transitions.
3. Carvedilol and the amino acid form one homogeneous phase through an ideal ratio and no component is in excess. In this case the thermal behaviour might be different from the behaviour of carvedilol and the amino acid.

Figure 2.8 represents DSC thermograms of Car-Phe and Car-Trp mixtures at 3:1, 1:1 and 1:3 molar ratios. Figure 2.8.a shows that depending on the molar ratio, the thermal behaviour of ball-milled Car-Phe mixtures is different. Firstly, the glass transitions occur at higher temperatures when carvedilol is in a higher proportion in the mixture. This is unexpected according to the theory (e.g., Gordon-Taylor equation, see section 1.4, [73, 74]), where the T_g of the mixture is expected to be higher when the component with the higher T_g is in greater proportion. In this case, the T_g of carvedilol amorphous being 35.65 °C and the T_g of Phe being 67.14 °C (appendix A.2), the result is unexpected. This may be interpreted through the subsequent recrystallisation behaviour. The recrystallisation occurs immediately after the T_g when Phe is in a higher proportion in the mixture. The two events (T_g and recrystallisation) are spaced out when carvedilol is in a higher proportion. Furthermore, either one or two recrystallisations occur depending on the ratio. For Car-Phe 3:1 and 1:1, two recrystallisations are observable. The subsequent melting points are consistent with carvedilol form III (hemihydrate) and form II. Regarding Car-Phe 1:3, only one recrystallisation occurs and corresponds to carvedilol form II (appendix A.3).
The T_gs of Car-Trp mixtures (figure 2.8.b) are in line with expectations since they are higher when Trp (T_g=72.92 °C) is in higher amount in the mixture. All the Car-Trp mixtures have only one T_g and one visible crystallisation event, which is intense for Car-Trp 1:3 mixture. This intense recrystallisation is similar to the recrystallisation of amorphous Trp (appendix A.2, figure A.2.4) albeit its not happening at the same temperature. Two melting events are visible when carvedilol is in higher proportions in the mixture, likely corresponding to the melting of carvedilol hemihydrate and carvedilol form II.

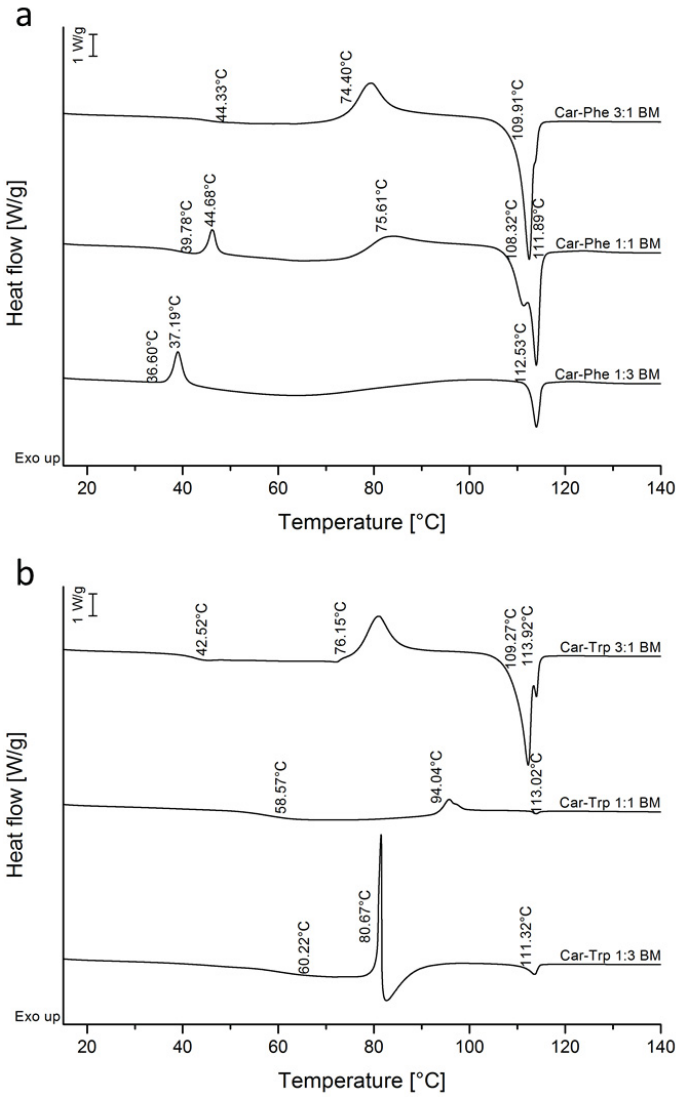


Figure 2.8. DSC thermograms of ball-milled Car-Phe (a) and Car-Trp (b) at 3:1, 1:1 and 1:3 molar ratios.

The observed thermal behaviour of Car-Phe and Car-Trp mixtures is not in line with the expectations. None of the three scenarios were entirely fulfilled. The formation of two coexisting amorphous phases, one homogeneous and the other containing the component in excess, was not demonstrated. Effectively, a single T_g was systematically observed although two distinct T_gs had been expected. However, it is speculated that the mixtures were not totally homogeneous and only weak interactions between the molecules were present. However, when carvedilol was in higher proportion in the mixture, the dominant thermal behaviour was likely the one of carvedilol. This is consistent with the high similarity observed between the DSC thermograms of Car-Phe 3:1 and Car-Trp 3:1 (figure 2.8). When the amino acid (Phe or Trp) is in higher proportion the thermal behaviour is noticeably different, suggesting an influence of the amino acid. Generally, the amino acid seems to have an influence on the thermal behaviour of the mixture, especially on the temperature at which the glass transitions and the crystallisations occur. Considering that a high T_g (higher than ambient temperature) is desirable for a better stability, Car-Trp mixtures have advantageous properties. Furthermore, Car-Trp 1:3 presents the highest T_g, and can hence be

considered the best ratio.

Thermal behaviour of carvedilol ball milled with acidic and basic amino acids at different ratios

DSC measurements were performed on carvedilol ball-milled mixtures with either basic or acidic amino acids at different molar ratios. As they were all partially amorphous (see section 2.2.1), a glass transition is still visible on the thermograms.
Figure 2.9 represents the thermorgams of carvedilol ball-milled mixtures with either arginine, glutamic acid or aspartic acid at 3:1, 1:1 and 1:3 molar ratios.
Arg was used as a basic amino acid for the co-amorphisation with carvedilol. Figure 2.9.a represents the DSC thermograms obtained for the three tested ratios. Firstly, the T_gs are close to the T_g of amorphous carvedilol (35.65 °C) or lower for Car-Arg 1:3 ball-milled mixture. This is due to the T_g of Arg itself, which was measured at 31.73 °C (appendix A.2, figure A.2.2). The fact that Arg has a lower T_g than the T_g of carvedilol is disadvantageous for the stabilisation of the co-amorphous mixture. The aim of adding an amino acid for the co-amorphisation is, among others, to increase the T_g of the obtained mixture. Secondly, after the glass transition either one or two recrystallisation occur. A particular endothermic event occurs at 82.43 °C for the Car-Arg 1:3 mixture, which corresponds to water evaporation and recognisable by its shape and temperature. This might suggest that arginine is hygroscopic as this is only observed when arginine is in excess in the mixture. The following melting events have temperature corresponding either to carvedilol hemihydrate (Car-Arg 1:1 and 1:3) or carvedilol form II.
Figure 2.9.b represents the DSC thermograms of Car-Glu ball-milled mixtures at 3:1, 1:1 and 1:3 molar ratios, Glu being an acidic amino acid. Car-Glu 3:1 and 1:1 both have a T_g close to that of amorphous carvedilol (T_g=35.65 °C). Car-Glu 1:3 has, on the contrary, a higher T_g. There is also an endothermic event occurring at 70.60 °C which is not clearly identified. It may be water evaporation from the sample, nevertheless, the shape is not representative of a typical water evaporation event and the temperature is lower than expected for such an event. Another possible interpretation is the association of this transition with the glass transition of glutamic acid (measured T_g for Glu BM 77.22 °C, appendix A.2, figure A.2.5). There would thus be two amorphous phases, one containing Glu amorphous in excess and one containing a homogeneous and co-amorphous Car-Glu mixture with a T_g of 49.89 °C. Furthermore, either one or two recrystallisation peaks are visible depending on the molar ratio of each Car-Glu mixture and eventually either one or two melting points. The melting signals of the crystalline forms are again consistent with the properties of carvedilol hemihydrate and carvedilol form II.

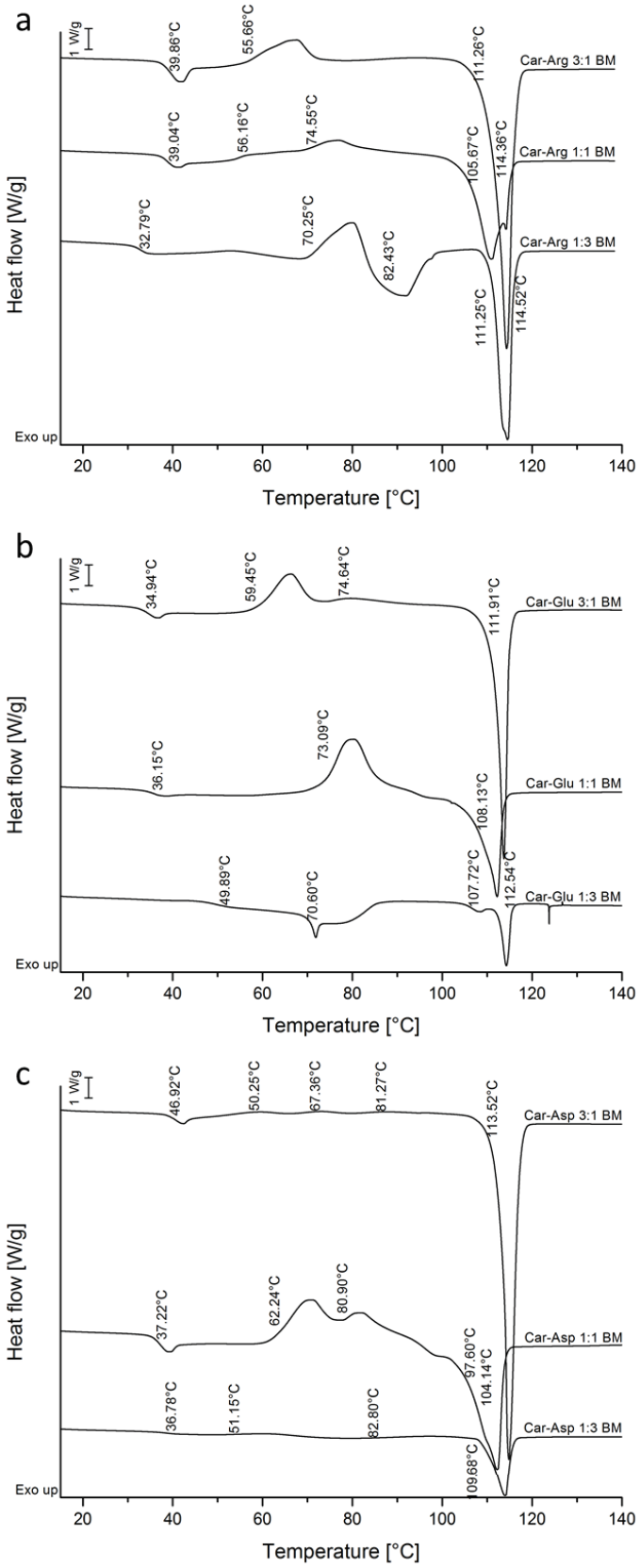


Figure 2.9. DSC of ball-milled Car-Arg (a), Car-Glu (b) and Car-Asp (c) at 3:1, 1:1 and 1:3 molar ratios.

Figure 2.9.c shows the DSC thermograms of Car-Asp ball-milled mixtures at 3:1, 1:1 and 1:3 molar ratios. Firstly, the measured T_gs are slightly higher than the T_g of carvedilol (35.65 °C). As the T_g of Asp is not easily measurable (appendix A.2, figure A.2.6), the influence of the amino acid on the T_g of the mixture is difficult to appreciate. Secondly, the crystallisation behaviour of the different mixtures differs strongly. The thermograms display two or three crystallisations which may correspond to either carvedilol recrystallisation or aspartic acid recrystallisation. Finally, the melting peaks are consistent with a mixture of carvedilol form III (hemihdrate) and form II, when the peak is wide or double, or to carvedilol form II when the melting peak is single and occurs at approximately 113.5 °C.

To conclude, the thermal behaviour of co-amorphous mixtures does not support the initial hypotheses formulated in page 22 regarding the impact of the carvedilol-amino acid ratio. The thermal behaviour of these mixtures is more complex than expected. However, a constant general behaviour is noticed specifically when carvedilol is in excess in the mixture, since similarities are observed in terms of recrystallisation and melting temperature. The amino acid can influence the thermal behaviour of the mixture negatively in specific instances, Car-Arg mixtures where the T_g was lowered below the T_g of amorphous carvedilol. DSC measurements on partially amorphous samples provided complementary information, and showed that these amino acids (Arg, Glu and Asp) do not bring any significant advantage for the stability of amorphous carvedilol.

Comparison between measured T_gs and calculated T_gs

The Gordon-Taylor equation is the most commonly used equation to predict the T_g of co-amorphous mixtures (section 1.4) [66, 73]. For this purpose, the density of the pure amorphous substances were needed. Considering the complexity of the amorphisation process, the resulting instability and the difference in density and quality of the powders obtained from the different amorphisation methods, it was decided to use the gas density of pure crystalline substances for the calculation. Density measurements were hence performed with helium pycnometry (method in section 3.2.7).
Theoretical T_gs were calculated using the Gordon-Taylor equation (equation 1.1 from section 1.4) for all the mixtures and Car-AA ratios. The results obtained are presented in figure 2.10 and compared with the T_gs measured by DSC. For each carvedilol-amino acid mixture, as shown in figure 2.10, the solid line indicates the measured T_g of pure amorphous carvedilol and the dashed line the measured T_g of pure amorphous amino acids, except for Car-Asp mixtures. The T_g of pure amorphous aspartic acid could not be determined due to the unsuccessful amorphisation using the described methods. Consequently, the theoretical T_g could not be calculated with the Gordon-Taylor equation, which requires the T_g of the pure amorphous substances composing the mixture.
The Gordon-Taylor equation does not account for the potential presence of unquantified adsorbed water, which can influence the resulting T_g, or any intermolecular interactions. In both cases the predicted T_g may thus significantly differ form the measured T_g. The potential shift in T_g due to intermolecular interactions may thus be useful in the determination of the ideal ratio in a co-amorphous mixture [64]. It is assumed that the ratio having the measured T_g which differs the most from its

calculated T_g exhibits the strongest intermolecular interactions [64]. Therefore, this ratio should be the ideal ratio for the targeted co-amorphous system.

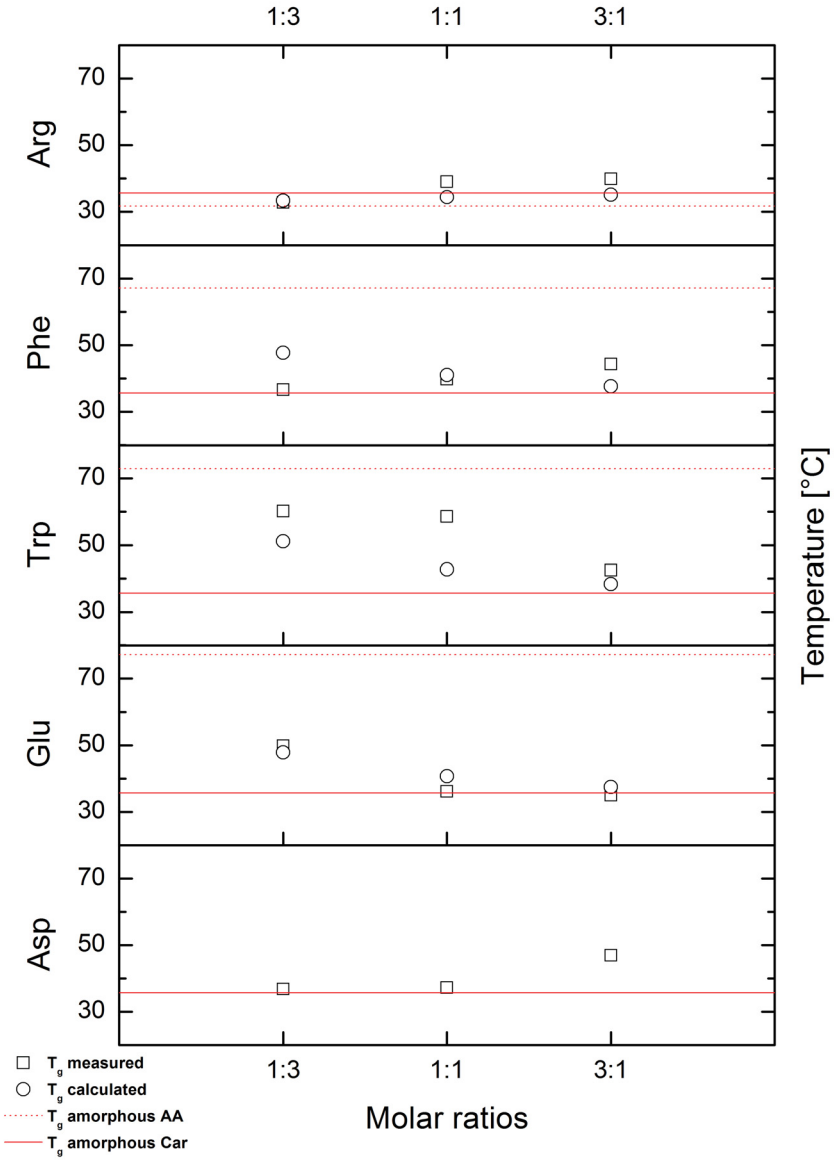


Figure 2.10. Calculated T_gs with Gordon-taylor equation and measured T_gs for Car-Arg, Car-Phe, Car-Trp, Car-Glu and Car-Asp mixtures at molar ratios 1:3, 1:1 and 3:1. The solid line indicates the measured T_g of pure amorphous carvedilol and the dashed line the measured T_g of the corresponding pure amorphous amino acid.

Among the Car-AA mixtures (figure 2.10), only Car-Trp mixtures show an obvious shift between the theoretical and measured T_gs. Car-Trp 1:1, in particular, exhibits a significantly higher measured T_g compared to the calculated T_g. Car-Phe mixtures show smaller shifts in T_gs, especially for the 1:3 ratio. However, the fact that the measured T_g is lower than the calculated T_g is expected to impact the stability of the mixture negatively. However, the lower T_g is not necessarily caused by intermolecular interactions, which are generally expected to increase the measured T_g.

Thermal behaviour of carvedilol-aspartic acid 1:1 ball milled and freeze dried

The thermal behaviour of ball-milled and freeze-dried carvedilol-aspartic acid 1:1 was measured by DSC (figure 2.11). Their XRPD patterns indicate partial amorphisation except for Car-Asp 1:1 freeze dried two times as shown in figure 2.6.

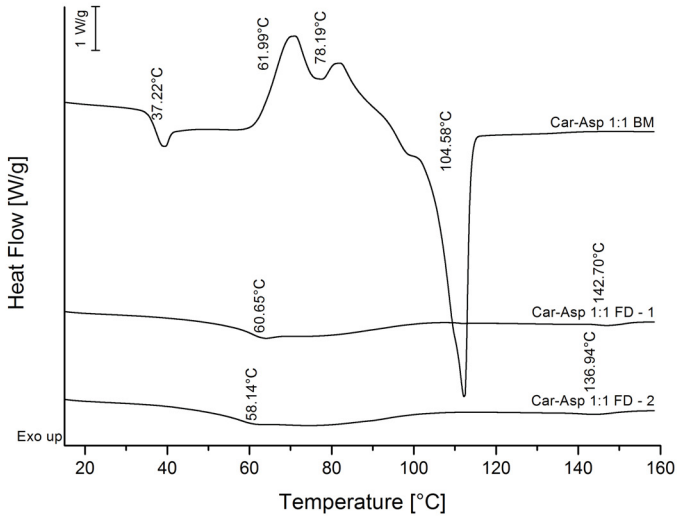


Figure 2.11. DSC thermograms of ball-milled (BM), and freeze-dried (FD) Car-Asp at 1:1 molar ratio. FD - 1: Car-Asp 1:1 freeze dried one time, FD - 2: Car-Asp 1:1 freeze dried two times

The thermal behaviours of the ball-milled mixture and the freeze-dried mixtures differ considerably from each other. This difference is unlikely to be caused exclusively by residual crystallinity given that Car-Asp 1:1 FD-1 also contained some level of residual crystallinity. In addition, Car-Asp 1:1 freeze dried one time or two times exhibits a similar thermal behaviour. The T_g of both mixtures is significantly higher than Car-Asp 1:1 BM. This suggests a difference in terms of intermolecular interactions between carvedilol and the aspartic acid and a more homogeneous co-amorphous mixture. Furthermore, the freeze-dried mixtures do not show any obvious recrystallisation or melting of carvedilol. This reflects a better stability of these mixtures when heated. However, a first endothermic event is observed after the glass transition which corresponds to the evaporation of water. This was expected due to the freeze drying process which implies an initial dissolution in water before the samples are frozen and eventually dried. A second endothermic event occurs at 143 °C or 137 °C (for Car-Asp 1:1 FD-1 and FD-2 respectively), neither of which corresponds to the melting temperature of any known carvedilol polymorphs described in the literature. Theses endothermic events, assuming they are melting events, occur at a temperature too high to correspond to the melting point of any known form of carvedilol and a temperature too low to correspond to the melting point of aspartic acid (decomposition at > 300 °C). A plausible interpretation is that carvedilol and aspartic acid form a co-amorphous salt, which shows a better stability under heating and is associated with its own melting temperature. This needs to be further investigated via other characterisation techniques to assess the type of interactions between carvedilol and aspartic acid.

2.2.3. Evaluation of interactions between the drug and the amino acid

Intermolecular interactions in the solid state can be analysed with a range of spectroscopic methods. The mostly used methods for the characterisation of medicinal substances are solid state nuclear magnetic resonance (ssNMR), Raman, near infrared (NIR), infrared (IR) and terahertz pulsed spectroscopy (TPS) [10].
ATR-FTIR and classical NMR were used for the analysis of co-amorphous mixtures with carvedilol as a drug. In ATR-FTIR spectroscopy, intermolecular vibrations are probed, for instance hydrogen bonding and band broadening caused by the amorphous sate. Classical NMR measurements using the appropriate solvent can also provide information on strong intermolecular interactions, such as salt formation. Deuterated dimethyl sulfoxide (DMSO), an aprotic solvent, was used to detect potential salt formation in the amorphous state. The aprotic character of the solvent used for solubilising the samples hinders the potential interactions involving labile hydrogens between the drug and the solvent. This approach enables the detection of the original strong interactions between the drug and the amino acid involving labile hydrogens, such as a salt formation. The atoms involved in the interaction exhibit generally broadened peaks in the NMR spectrum.

ATR-FTIR analysis of ball-milled mixtures

The successfully amorphised mixtures Car-Phe and Car-Trp were analysed using FTIR at molar ratios 3:1, 1:1 and 1:3. The FTIR spectra of Carvedilol and either Phe or Trp in a crystalline form were used as references. Car-Arg, Car-Glu and Car-Asp ball-milled mixtures were not completely amorphous as shown in figure 2.3. Nevertheless, their FTIR spectra will be presented for comparison except for Car-Arg were the effect of adsorbed water in the sample affected the quality of the spectra.
A wavenumber range of 3500 to 2500 cm^{-1} was selected, where the frequencies of infrared absorptions of the most relevant functional groups appear. Based on the functional groups within carvedilol (figure 2.12), interactions with the amino acid are considered at the secondary amine site, at the free hydroxyle site and through π-π interactions. However, the secondary amine present in the carbazole group – which exhibits no basic character – and in the hydroxyle group are not expected to be involved in potential salt formation. Nevertheless, hydrogen bonds are possible through all hydrogen donors or acceptors except for the lone pair of electrons of the nitrogen atom in the carbazole group. This lone pair of electrons is involved in the aromatic character of the heterocycle. A detailed analysis of FTIR spectra will be made, with a particular focus on the N-H stretching signal from the secondary amine (aliphatic central chain).

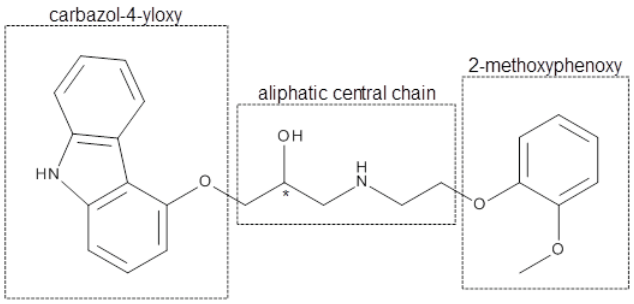


Figure 2.12. Chemical structure of carvedilol.

Car-Phe ball-milled mixtures FTIR spectra of the ball-milled Car-Phe mixtures at different ratios are represented in figure 2.13. The FTIR spectrum of carvedilol crystalline exhibits two important signals, both corresponding to N-H stretching of secondary amines. The first signal at 3398 cm^{-1} represents the N-H stretch of the secondary amine of the carbazole group (figure 2.12). The second signal, at 3342 cm^{-1}, corresponds to the N-H stretch of the secondary amine of the aliphatic central chain. The latter is likely to disapear or be modified when carvedilol and the amino acid interact through hydrogen bonding or as a result of salt formation.
The three spectra of the co-amorphous mixtures of Car-Phe show broadened signals. This effect is expected and manly due to the amorphous state of the samples. With higher proportions of Phe in the mixture, the signals characteristic of the IR absorption of carvedilol tend to weaken and those of Phe to strengthen. However, the signal at 3342 cm^{-1} is weak in all three spectra. Therefore, it is possible to assume that intermolecular interactions are present and may be hydrogen bonds between the secondary amine and hydrogen donors and acceptors from Phe. Another possible interaction is between the carvedilol molecules themselves. Nevertheless, when the spectra of carvedilol crystalline and carvedilol amorphous are compared, this signal appears broadened and weak only in the amorphous state. In the case of the co-amorphous mixtures, the peak broadening is likely caused by the amorphous nature of the substance rather than resulting from intermolecular interactions between carvedilol molecules.

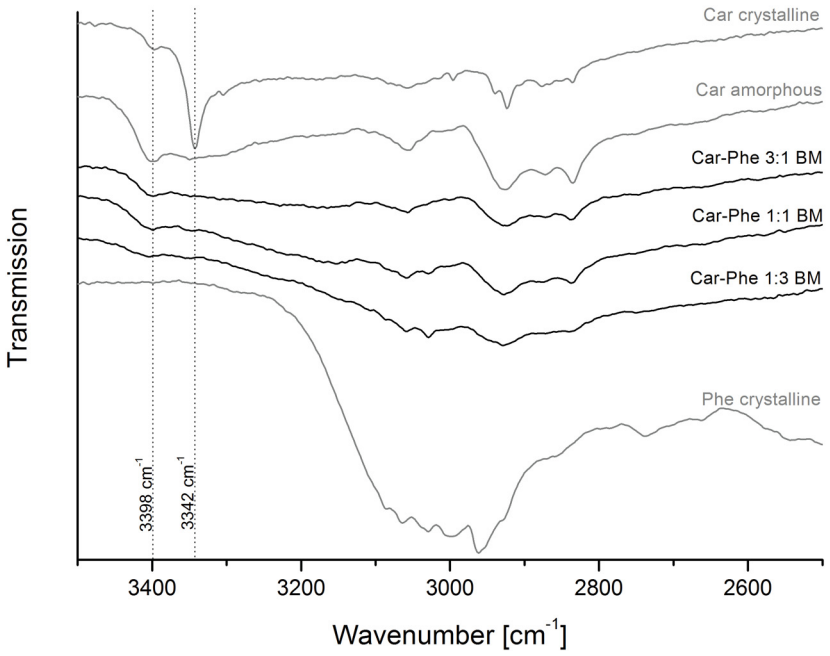


Figure 2.13. FTIR spectra of Car-Phe ball-milled mixtures (ratios 3:1, 1:1 and 1:3), melt-quenched carvedilol (amorphous), carvedilol crystalline and L-phenylalanine crystalline.

Car-Trp ball-milled mixtures Car-Trp ball-milled mixtures were successfully amorphised as shown in figure 2.3. FTIR measurements were performed on the different mixtures at ratios 3:1, 1:1 and 1:3. The obtained spectra are represented in figure 2.14. Similarly to Car-Phe ball-milled mixtures, particular attention is given to potential interactions with the secondary amine of the aliphatic central chain. This is expected to modify the N-H stretch signal at $3342\,\mathrm{cm}^{-1}$. The spectra from the Car-Trp mixtures exhibit also a gradual change in the intensity of the signals depending on the mixture composition. The spectrum representing the mixture with the higher amount of carvedilol shows a strong similarity with the spectra of ball-milled carvedilol. The opposite is true when L-tryptophan is in higher proportion in the mixture, characteristic signals for Trp appear. As previously mentioned, the signal change at $3342\,\mathrm{cm}^{-1}$ is more likely caused by the amorphous nature of the substance rather than intermolecular interactions such as hydrogen bonds.

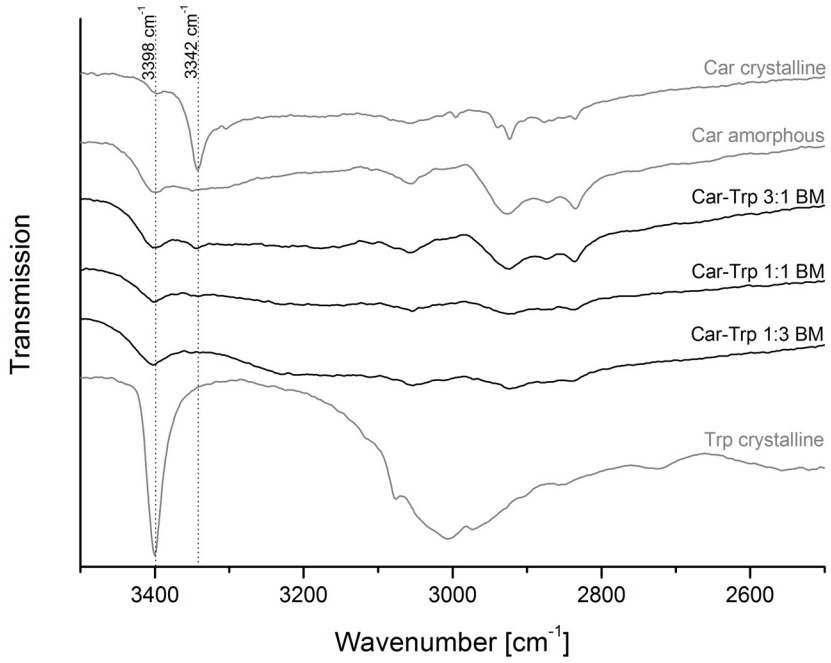


Figure 2.14. FTIR spectra of Car-Trp ball-milled mixtures (ratios 3:1, 1:1 and 1:3), ball-milled carvedilol (partially amorphous), carvedilol crystalline and L-tryptophan crystalline.

Car-Glu ball-milled mixtures Car-Glu mixtures were still exhibiting residual crystallinity after ball milling, the amorphisation was thus not totally successful as shown in figure 2.3. Nonetheless, FTIR measurements were performed in order to detect potential intermolecular interactions and better understand their mechanisms. The represented FTIR spectra in figure 2.15 correspond to Car-Glu ball-milled mixtures at ratios 3:1, 1:1 and 1:3. Each mixture exhibits a strong signal from the N-H stretching of the secondary amine located on the aliphatic central chain (figure 2.12) at $3342\,\mathrm{cm}^{-1}$. Several interpretations are suggested. Firstly, this may mean that carvedilol is not interacting through hydrogen bonds at the location of the secondary amine functional group. The signal at $3342\,\mathrm{cm}^{-1}$ decreases in intensity as the amount of Glu increases in the mixture, as anticipated. Secondly, it is possible that carvedilol was physically unstable to such an extent that it has crystallised in the mixture, in spite of the fact that the FTIR measurements were performed within the next two days of ball milling. A combination of both root causes is also plausible. On the basis of the previous characterisation results (XRPD and DSC), the combination between carvedilol and L-glutamic acid is not the most successful pair. L-glutamic acid is not considered as a good co-former for co-amorphisation. As revealed by FTIR measurements, co-amorphisation is hindered by insufficient intermolecular interactions between Glu and carvedilol.

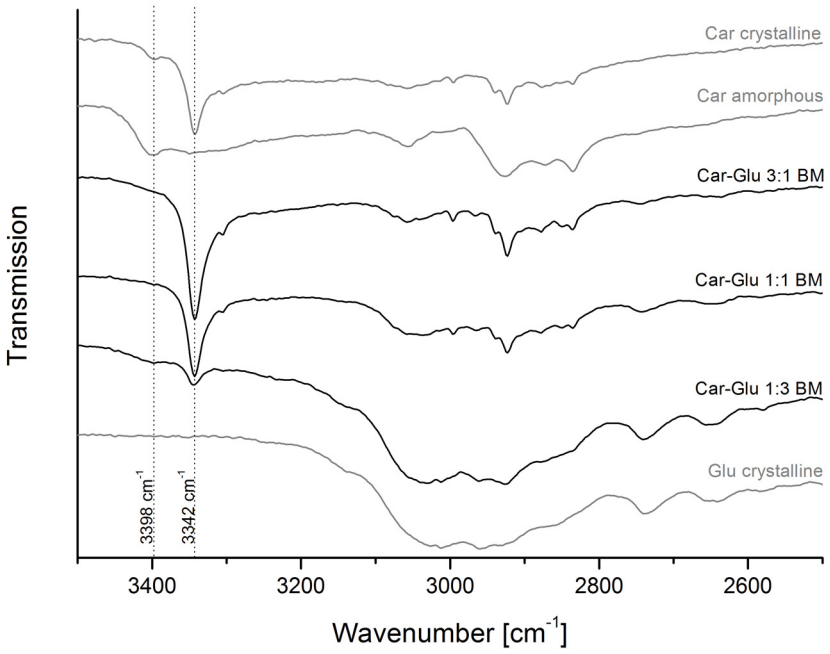


Figure 2.15. FTIR spectra of Car-Glu ball-milled mixtures (ratios 3:1, 1:1 and 1:3), ball-milled carvedilol (partially amorphous), carvedilol crystalline and L-glutamic acid crystalline.

Car-Asp ball-milled mixtures Car-Asp ball-milled mixtures were not totally amorphised as shown in figure 2.3. All three ratios were partially amorphous (figure 2.5). Figure 2.16 represents FTIR spectra of Car-Asp ball-milled mixtures at 3:1, 1:1 and 1:3 molar ratios. Similarly to Car-Glu mixtures, the characteristic signals of carvedilol weaken as the amount of Asp increases in the mixture. The FTIR spectrum of Car-Asp 3:1 mixture exhibits a stronger peak at $3342\,\mathrm{cm}^{-1}$ compared to the other ratios and carvedilol amorphous. The signal intensity is comparable to that of carvedilol crystalline. This is consistent with the fact that this mixture exhibited residual crystallinity and is probably unstable, resulting in higher amount of crystalline carvedilol contributing to the $3342\,\mathrm{cm}^{-1}$ signal. Broadened signals caused by the partially amorphous nature of the mixtures are observed for all three spectra and characteristic signals of L-aspartic acid tend to appear as the amount of amino acid increases. Once more, this data does not highlight significant interactions between carvedilol and the amino acid.

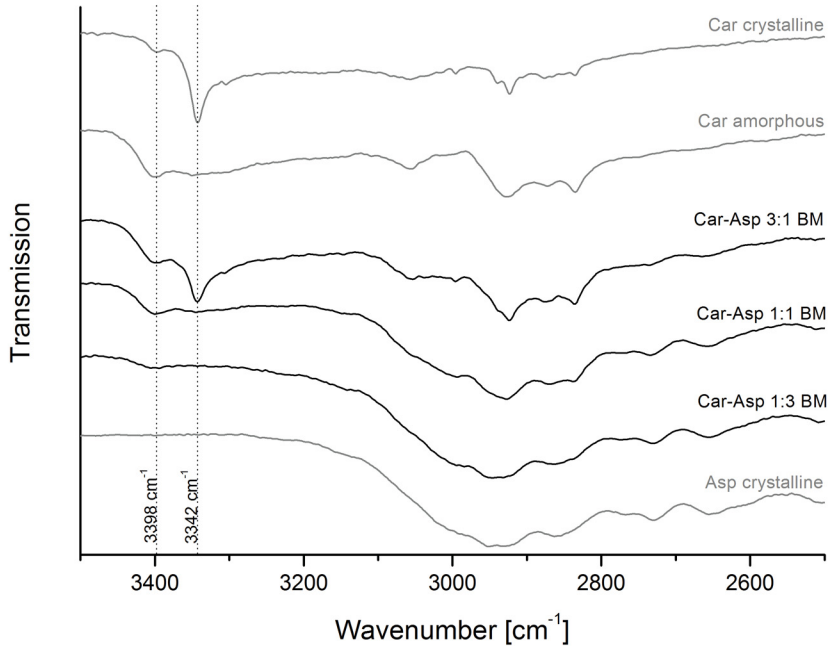


Figure 2.16. FTIR spectra of Car-Asp ball-milled mixtures (ratios 3:1, 1:1 and 1:3), ball-milled carvedilol (partially amorphous), carvedilol crystalline and L-aspartic acid crystalline.

Car-Asp 1:1 freeze-dried mixture In this section, a comparison between FTIR spectra of Car-Asp 1:1 ball-milled and freeze-dried mixtures is presented. Previous experiments by XRPD and DSC highlighted differences in the structural and thermal properties of the mixtures. In addition, the mixtures display dissimilar macroscopic properties and as a result contrasting powder quality. It is therefore of interest to investigate the nature of the intermolecular interactions to understand how the amorphisation process might differ. Figure 2.17 represents FTIR spectra of ball-milled and freeze-dried Car-Asp 1:1 mixtures. As mentioned in previous sections, the freeze-dried mixture was amorphised successfully whereas the ball-milled mixture was only partially amorphous. More broadened signals are hence expected in the spectrum of the freeze-dried mixture. Unexpectedly, the signals are also lower in intensity. This tend is observed for all carvedilol signals, such as secondary amine signals and aliphatic C-H stretch and bend signals between $3000\,\mathrm{cm}^{-1}$ and $2800\,\mathrm{cm}^{-1}$. In comparison with the FTIR spectra of crystalline L-aspartic acid, the spectrum of freeze-dried Car-Asp 1:1 mixture does not exhibit obvious characteristic signals from the amino acid. However, the signals in the spectrum of Car-Asp 1:1 freeze dried are relatively weak, which might imply intermolecular interactions. The disappearance of the signal at $3342\,\mathrm{cm}^{-1}$ is consistent with the presence of at the least a hydrogen bond with the secondary amine of the aliphatic central chain, if not an even stronger interaction such as ionic bonding.
This difference observed between both spectra demonstrates on the one hand that the process of amorphisation may influence the intermolecular interactions. On the other hand, it shows that intermolecular interactions can be detected by FTIR in the amorphous state, particularly when reference spectra are available for comparison.

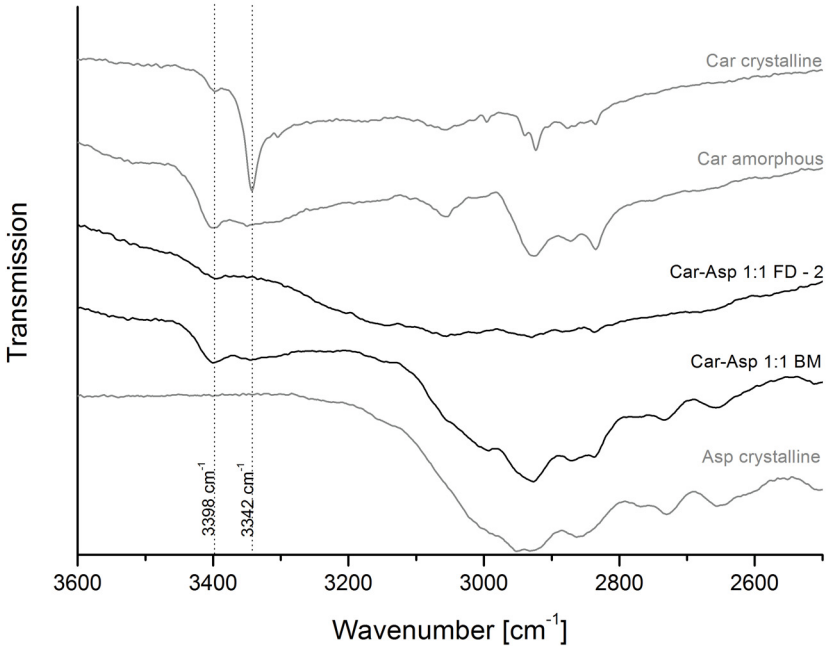


Figure 2.17. FTIR spectra of Car-Asp freeze-dried mixture, ball-milled mixture, partially amorphous carvedilol (ball-milled), carvedilol crystalline and L-aspartic acid crystalline.

NMR analysis of Car-Asp 1:1 co-amorphous mixture

NMR analyses were performed on the successfully amorphised mixtures, ball-milled Car-Phe 1:1, ball-milled Car-Trp 1:1 and freeze-dried Car-Asp 1:1 mixtures. During the dissolving step in deuterated DMSO, a difference in solubility was frequently observed between the mixtures. Car-Asp 1:1 freeze-dried mixture exhibited a small amount of undissolved powder whereas Car-Phe and Car-Trp were fully dissolved. Nonetheless, the measurement was performed with the assumption that L-aspartic acid was probably not fully in solution due to its poor solubility in DMSO. The obtained spectra were compared to the spectra of crystalline carvedilol and crystalline amino acids except for L-aspartic acid, which was insoluble in DMSO.
The spectra of Car-Phe and Car-Trp were identical to the spectrum of crystalline carvedilol and the amino acid combined. Consequently, only the spectrum of Car-Asp 1:1 is represented in figure 2.18. The absence of changes in the signals shows that there were no strong intermolecular interactions between carvedilol and either L-phenylalanine or L-tryptophan, such as ionic bonds. This result consolidates the assumption that for both amino acids no strong interactions are expected. Moreover, since the measurement takes place in solution (deuterated DMSO) weak interactions are not expected to be detectable.
The NMR spectrum of Car-Asp 1:1 exhibits two major changes in comparison to the NMR spectrum of crystalline carvedilol (figure 2.18): (1) The majority of signals are slightly shifted, (2) the peak from the N-H secondary amine at approx 5.2 ppm is broadened and the peak at 3.3 ppm corresponding to residual water is also broadened. The signal from the secondary amine of the aliphatic central chain changed

from a doublet corresponding to 1 hydrogen, to a broad singlet corresponding to 2 hydrogens. This change suggests that the secondary amine from the aliphatic central chain is basic and can exchange protons with water. This basic property and the slight shifts in signal imply that carvedilol formed a salt with L-aspartic acid in the solid state. A third change also occurred, namely the disappearance of the hydrogen signal of the hydroxyl group at 2.0 ppm. This disappearance is frequent when -OH groups can interact with water present in the sample in solution.

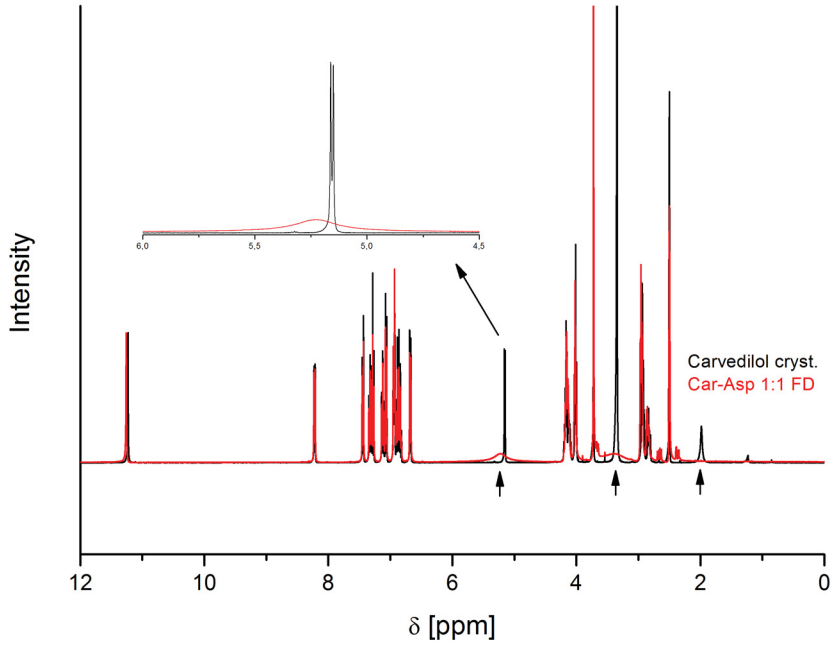


Figure 2.18. NMR spectra of Car-Asp 1:1 (red) and carvedilol crystalline (black) in deuterated DMSO.

NMR measurements of co-amorphous mixtures in aprotic solvent such as deuterated DMSO demonstrate the potential of NMR in highlighting potential salt formation. While FTIR can reveal many types of intermolecular interactions such as hydrogen bonds and weak interactions. It can be challenging to differentiate strong interactions such as salt formation from weaker interactions, especially without adequate references for comparison. Therefore, NMR and FTIR both provide useful and complementary information.

2.2.4. Influence of the hygroscopicity of the substances on the co-amorphous mixtures

It is reasonable to assume that the hygroscopicity of the used substances may be an important factor influencing the co-amorphous system [77]. The water content may interfere with the amorphisation process and alter the co-amorphous state. Dynamic vapour sorption analysis (DVS) was used for the determination of water content of crystalline material. This experiment was difficult to reproduce on co-amorphous material since the amorphous state is intrinsically unstable in such stress conditions of humidity and duration. The results would not reflect the real hygroscopicity of the co-amorphous mixture but that of another form appearing during the experiment, for example a crystalline form or even a form derived from a decomposition product. Accordingly, it is considered more relevant to find a relationship between the hygroscopicity of the crystalline materials used before amorphisation and the final outcome in terms of success of amorphisation and physical stability. This relationship would give precious information on the potential behaviour of the co-amorphous mixtures and help in the selection of the right amino acid co-former.
Following the DVS analysis of carvedilol and amino acids, a classification could be established (table 2.2) in accordance with the criteria of the European Pharmacopoeia 9.0/5.11. A majority of the used crystalline substances were considered to be non hygroscopic. However, L-arginine is very hygroscopic and L-phenylalanine slightly hygroscopic. These results may explain a number of characteristic properties previously observed on the mixtures.
The high hygroscopic level of L-arginine is consistent with the unsuccessful amorphisation by ball milling. The XRPD pattern of the mixtures containing L-arginine exhibited characteristic signals of L-arginine and also of carvedilol. It was the only pair of drug-AA where residual crystallinity of carvedilol was present after ball milling. This could be explained by the probable presence of water in the mixture due to the ability of L-argnine to attract a significant amount of water. During ball milling, the water increases the molecular mobility and lowers the T_g of the mixture, which in turn increases the risk of recrystallisation and hinders a potential amorphisation at 5 °C. Consequently, the equilibrium between disrupting the crystal lattice by ball milling and the constant recrystallisation of the substances is shifted towards recrystallisation. The resulting sample was hence not completely amorphous.

Table 2.2. Water uptake of crystalline Car and AAs measured with dynamic vapour sorption and classification according Ph. Eur. 9.0/5.11.

Substance	Water uptake [%]	Classification (Ph. Eur.)
Carvedilol	0.04	non hygroscopic
L-arginine	20.59	very hygroscopic
L-phenylalanine	0.22	slightly hygroscopic
L-tryptophan	0.09	non hygroscopic
L-glutamic acid	0.04	non hygroscopic
L-aspartic acid	0.01	non hygroscopic

The ball-milled carvedilol-AA mixtures generally tend to recrystallise in two crystalline modifications, as previously observed. One of the crystalline modifications is a hemihydrate (form III) and the other is crystalline form II, an anhydrate. In the case

of mixtures containing Arg or Phe, this can be explained by the ability of both amino acids to attract water in the sample. However, the recrystallisation as a hemihydrate also occurred at specific ratios with the other amino acids (for instance Car-Trp 3:1, Car-Asp 1:1 and Car-Glu 1:1). The measurement of the substance hygroscopicity in their crystalline state is thus insufficient to fully explain this phenomenon. Alternatively, they may exhibit an increased hygroscopicity in their amorphous form. To test this hypothesis, carvedilol was melt quenched and thus obtained in its amorphous form. This sample was stored at ambient conditions of temperature and humidity ($(22.0 \pm 2.0)\,^{\circ}\mathrm{C}$ and $(55.0 \pm 10.0)\,^{\circ}\mathrm{C}$) for 8 months. Figure 2.19 represents the XRPD pattern of this melt-quenched carvedilol after 8 months of exposure to room conditions in comparison to carvedilol form II. The analysed pattern was identified as carvedilol form III, a hemihydrate. This implies that amorphous carvedilol itself attracts enough water to be able to recrystallise as a hemihydrate.

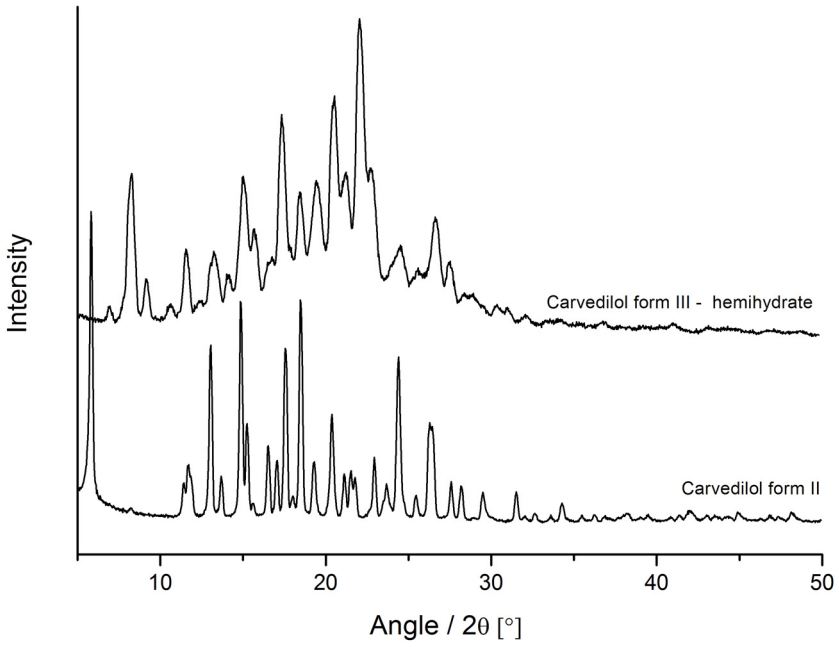


Figure 2.19. XRPD patterns of carvedilol recrystallised after amorphisation and carvedilol form II.

It was observed that a high water content of one of the components of the mixture can hinder a complete amorphisation of the mixture. Moreover, the hygroscopicity of the amorphous form can differ from the crystalline state. Amorphous carvedilol crystallises spontaneously at room conditions into a hemihydrate. Also depending on the Car-AA ratio, despite the presence of an amino acid in a co-amorphous state, carvedilol recrystallises partially into a hemihydrate.
The hygroscopicity of the used substances is obviously an important factor. Water content measurement is not only useful in the amorphous form but also in the crystalline form before the amorphisation process begins. Knowledge of the hygroscopicity is helpful to select a good co-former for the co-amorphisation and to adjust milling conditions, for example the milling temperature or the surrounding humidity during the amorphisation process.
The ability of amorphous carvedilol to spontaneously recrystallise into a hemihydrate over time when stored in room conditions shows that amorphisation could be used as a technique for obtaining hydrated forms of a substance. This technique can be used in addition to recrystallisation in various solvents for the screening of polymorphic forms in research.

2.2.5. Solubility of co-amorphous systems

It is reasonable to assume that co-amorphisation enhances the solubility of poorly soluble substances. Therefore, solubility measurements were performed on Car-AA mixtures showing complete or partial amorphisation. Both types of mixtures were used to determine whether the level of amorphisation is an important factor for solubility enhancement. Three mixtures with different amino acids were used for the

solubility testing, one basic (Arg), one neutral (Phe) and one acidic (Asp). Using these three amino acids it will be possible to observe whether the acidity of the co-former can influence the solubility of carvedilol, for example by slightly changing the pH of the solution.
Figure 2.20 presents the results of the solubility tests using carvedilol crystalline, carvedilol amorphous, Car-AA co-amorphous mixtures and physical mixtures (crystalline) at the same ratios. An arbitrary limit at 0.15 mg/ml is represented by a red line to facilitate comparisons.
The comparison of carvedilol crystalline and carvedilol melt quenched (amorphous) shows that they have a comparable solubility in 0.1M HCl. Similarly, carvedilol in all physical mixtures has an equivalent solubility. However, carvedilol in all ball-milled mixtures has a lower solubility, which does not exceed 0.15 mg/ml. This reduced solubility is however not significant after the analysis of variance (ANOVA) except for Car-Asp 1:1 BM ($p = 0.02$). The amorphous nature of the ball-milled mixtures is unlikely to be the only variable affecting their solubility, since the same trend of reduced solubility is not observed for other amorphous mixtures. This decrease in solubility is surprising and can be partially correlated to the amorphisation method. Ball-milled powders, as represented in section2.1, figure 2.1, exhibit irregular particle size and shape which may well have a negative impact on the solubility. Melt-quenched carvedilol, on the contrary, exhibits almost no difference in solubility and its particle size and shape were relatively homogeneous due to manual crushing after quenching. Another explanation may be that the final solubility of carvedilol is driven by another polymorph or solvatomorph which recrystallises in solution. The polymorph may not be the same when the sample is ball milled and hence exhibits a lower solubility.

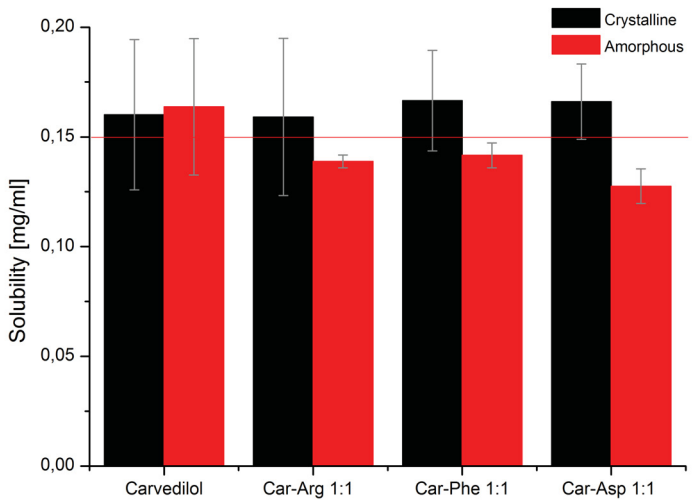


Figure 2.20. Solubility of carvedilol crystalline and melt quenched, Car-Arg 1:1, Car-Phe 1:1 and Car-Asp 1:1 as physical mixtures (crystalline) and ball-milled mixtures (amorphous) in 0.1 M HCl solution, ($\bar{x} \pm s, n = 3$).

In summary, this experiment shows that amorphisation does not enhance the solubility of carvedilol in 0.1 M HCl. Moreover, co-amorphisation with an amino acid by ball milling does not improve carvedilol solubility but rather tends to lower it. The type of amino acid and its acidity seem to play a minor role on the solubility of carvedilol

in 0.1 M HCl. Comparably, whether the samples are partially amorphous or totally amorphous has no obvious influence on the solubility as Car-Phe 1:1 was the only mixture totally amorphised after ball milling.

2.2.6. Intrinsic dissolution rates of co-amorphous systems

Co-amorphisation was shown not to improve the solubility of carvedilol. Therefore intrinsic dissolution tests were performed in order to assess whether co-amorphisation can be beneficial by increasing the intrinsic dissolution rate of carvedilol. Different amino acids as co-former are first tested in order to determine the influence of the type (acidity) of amino acid on the intrinsic dissolution rate of carvedilol. The influence of the ratio between carvedilol and the amino acid will subsequently also be tested.

Intrinsic dissolution rates of carvedilol-amino acid co-amorphous mixtures

The same co-amorphous mixtures as for the solubility testing were used: Car-Arg, Car-Phe and Car-Asp at 1:1 molar ratios. The mixtures consist of one basic amino acid (Arg) one neutral (Phe) and one acidic (Asp). The influence of the acidity of the amino acid on the intrinsic dissolution profile of carvedilol will be studied, as well as the influence of the level of amorphisation. Therefore, partially amorphous mixtures were included in the study such as Car-Arg and Car-Asp. As references, the respective physical mixtures are also used as well as carvedilol crystalline (form II) and carvedilol melt quenched (amorphous). All the measurements were performed in 0.1 M HCl.
Figure 2.21.a presents the intrinsic dissolution profiles of the different ball-milled mixtures, the respective physical mixtures and carvedilol form II and amorphous. Firstly, it is observed that Car-Asp as a ball-milled mixture and as a physical mixture have both a significantly higher released amount of carvedilol than all samples throughout the experiment. Therefore, a second similar figure (figure 2.21.b) is presented to allow an easier comparison of the intrinsic dissolution profiles of the other mixtures or substances. Car-Asp physical mixture exhibits a higher released amount of carvedilol than other co-amorphous or partially co-amorphous mixtures despite being completely crystalline. However, a better intrinsic dissolution profile is observed for this pair of substances in its partially co-amorphous form. Secondly, ball-milled Car-Phe, which was a completely co-amorphous system, exhibits a lower released amount of carvedilol than Car-Asp mixtures. Thirdly, carvedilol crystalline and carvedilol amorphous have an identical dissolution profile.
Figure 2.21.c presents the intrinsic dissolution rates (IDR) of all the tested samples. This rate is defined as the slope of the linear regression of the intrinsic dissolution profiles. Car-Asp mixtures have the two highest intrinsic dissolution rates. In addition, Car-Asp in a partially co-amorphous state has an intrinsic dissolution rate more than a two-fold higher compared to its crystalline form. The other mixtures release carvedilol at a significantly slower rate.

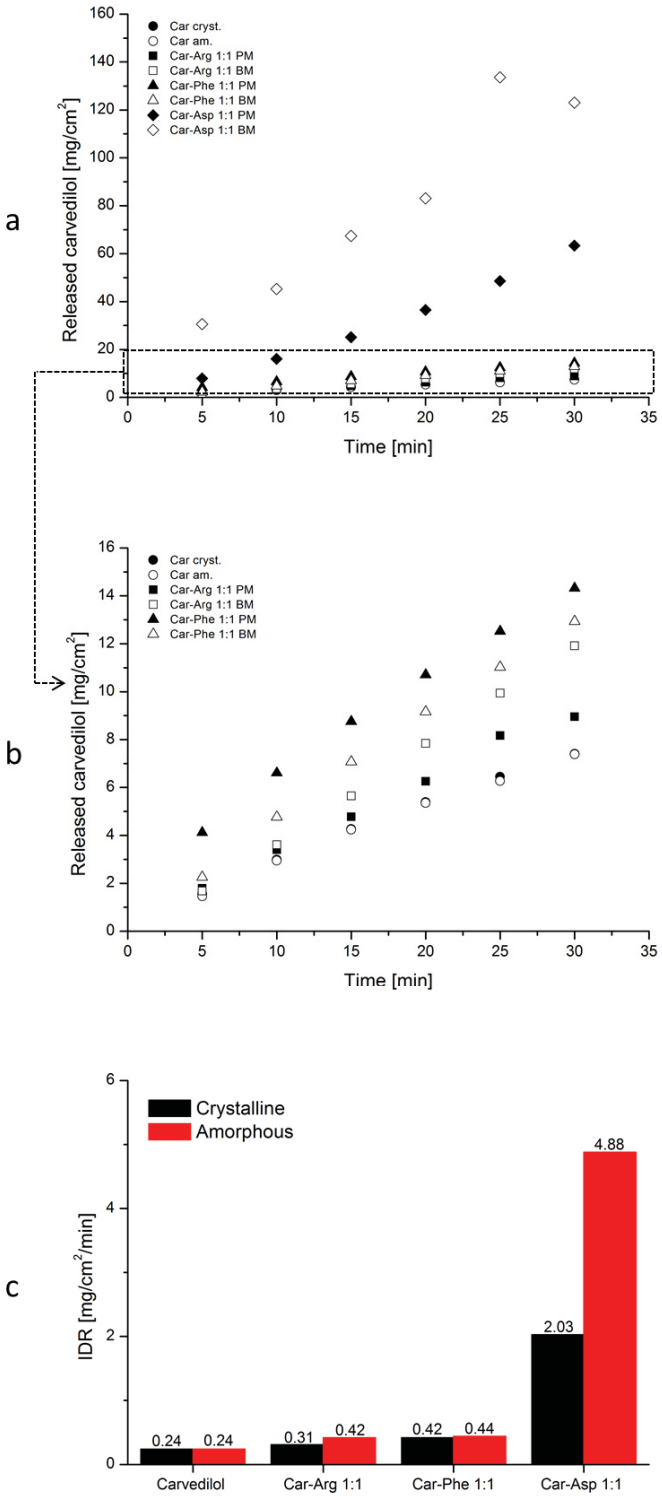


Figure 2.21. Intrinsic dissolution profiles in 0.1 M HCl of carvedilol crystalline and melt-quenched (am.), Car-Arg, Car-Phe, Car-Asp mixtures at 1:1 molar ratio as physical mixtures (PM, crystalline) and ball milled (BM) (a). Enlarged view of carvedilol crystalline and amorphous, Car-Arg 1:1, Car-Phe 1:1 PM and BM intrinsic dissolution profiles in 0.1 M HCl (b). Resulting intrinsic dissolution rates (IDR) for each sample (c); ($n = 3$).

In summary, it can be concluded that the IDR of carvedilol can be enhanced by pairing it with the appropriate amino acid. The acid-base properties of the amino acid used as a co-former have the most importance for increasing the IDR. The contribution of an acidic amino acid is likely derived from its interaction with carvedilol molecules in solution. The crystalline or amorphous nature of the mixture is also an important factor, regardless to the level of amorphisation. Car-Asp 1:1 partially amorphous exhibited a significantly higher IDR than in its crystalline counterpart. The level of amorphisation seems to play no significant role towards the IDR.

Intrinsic dissolution rates of Car-Asp at different ratios

As the most desirable IDR was observed for ball-milled Car-Asp 1:1, the study was subsequently focused on the influence of the carvedilol - Asp ratio on the IDR. Therefore, intrinsic dissolution measurements were performed in the same conditions as previously described using Car-Asp at 3:1, 1:1 and 1:3 molar ratios. These measurements are compared with the corresponding physical mixtures and the references carvedilol crystalline and melt quenched.

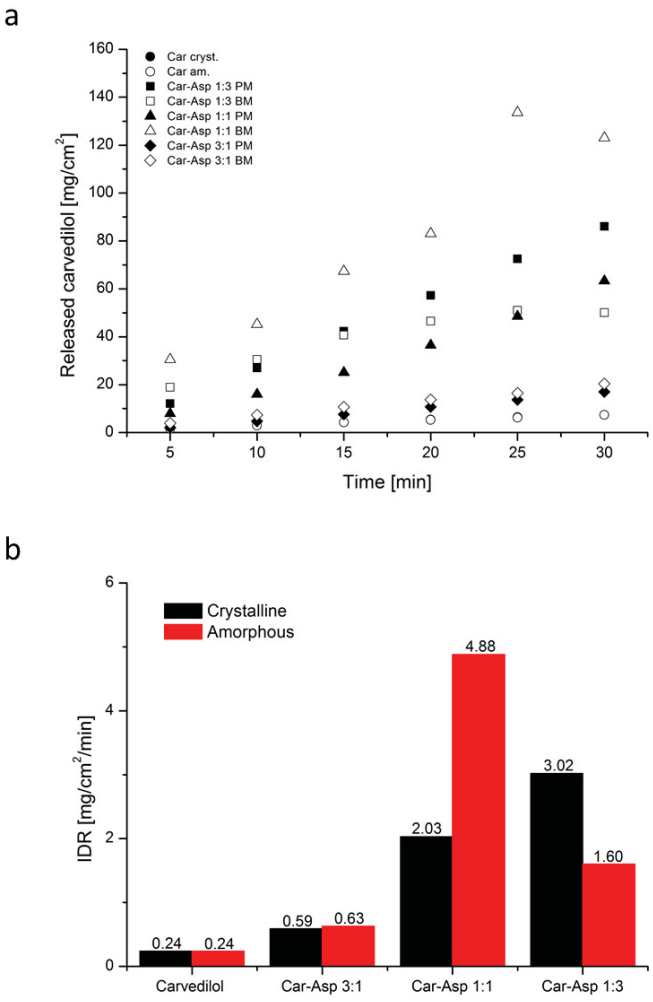


Figure 2.22. Intrinsic dissolution profiles in 0.1 M HCl of carvedilol crystalline and melt-quenched (am.), Car-Asp mixtures at 3:1, 1:1 and 1:3 molar ratios as physical mixtures (PM, crystalline) and ball milled (BM) (a). Resulting intrinsic dissolution rates (IDR) for each sample (b); ($n = 3$).

Figure 2.22 presents the intrinsic dissolution profiles of Car-Asp at the three molar ratios (a) and their intrinsic dissolution rates (b). All Car-Asp mixtures display a higher IDR than carvedilol alone. Regarding the Car-Asp physical mixtures, the IDR decreases as the amount of carvedilol increases in the mixture. On the contrary, in the co-amorphous state, the IDR is higher for the 1:1 molar ratio compared to the IDR for the 1:3 molar ratio. This difference suggests that Car-Asp 1:1 in a co-amorphous state is the most desirable ratio in terms of dissolution rate. The proposed interpretation is that 1:1 is closest to the ideal ratio allowing the most effective interaction between Carvedilol and L-aspartic acid. As observed in the previous experiments, Car-Asp 1:1 is able to form a co-amorphous salt. Car-Asp 1:3 in a co-amorphous state exhibits a lower IDR than for the corresponding physical mixture. In this case co-amorphisation did not show any benefit but rather a disadvantage.

Following the characterisation of ball-milled co-amorphous systems containing carvedilol and an amino acid in terms of solubility and intrinsic dissolution, it can be concluded

that co-amorphisation does not necessarily enhance the solubility of poorly water soluble drugs. This method may however enhance the intrinsic dissolution rate of the substance. An increase in the intrinsic dissolution rate would still be a benefit when formulating poorly soluble drugs, especially when targeting an immediate release tablet formulation.

2.2.7. Stability study of co-amorphous systems

Co-amorphous systems have to be physically stable in order to allow potential downstream processing and also long time storage. Therefore, a brief stability study was conducted. DSC was used as the main assessment analytical method in order to observe changes in thermal events over the storage duration. A particular focus will be given to glass transition temperatures (step change) and the number of melting events (endothermal events), which provide valuable information on recrystallised forms.
A stability study was conducted to test the stability of the co-amorphous systems. First, the stability of the co-amorphous systems Car-Phe and Car-Trp was studied using DSC. The influence of the amino acid on the physical stability is assessed through a comprehensive analysis of the observed thermal events.
The second part of the study consists in analysing the influence of the ratio between carvedilol and the respective amino acid on the physical stability in terms of thermal behaviour.
The third part of the stability study is focused on the influence of ambient humidity. Accordingly, different humidity conditions are selected and the influence of the storage conditions on the physical stability is assessed.
Since elevated temperatures are already known to alter the physical stability of amorphous systems [78], further stability testing at elevated temperature was not conducted.

Influence of the selected amino acid on the stability of the co-amorphous mixture

Two successfully amorphised co-amorphous systems were used for this part of the stability study, namely Car-Phe and Car-Trp at 1:1 molar ratio. Both mixtures were stored at dry conditions in order to analyse exclusively the influence of the selected amino acid over time on the thermal behaviour of the obtained co-amorphous mixtures. DSC measurements were performed after milling and subsequently after 7, 28, 56, 84 and 183 days of storage. In figure 2.23 the temperatures of the different thermal events are reported. Steps in DSC thermograms are described in terms of step changes (□) assumed to be glass transitions, exothermal events (◯) which are mostly recrystallisations, and endothermal events (△) which are mostly melting events.

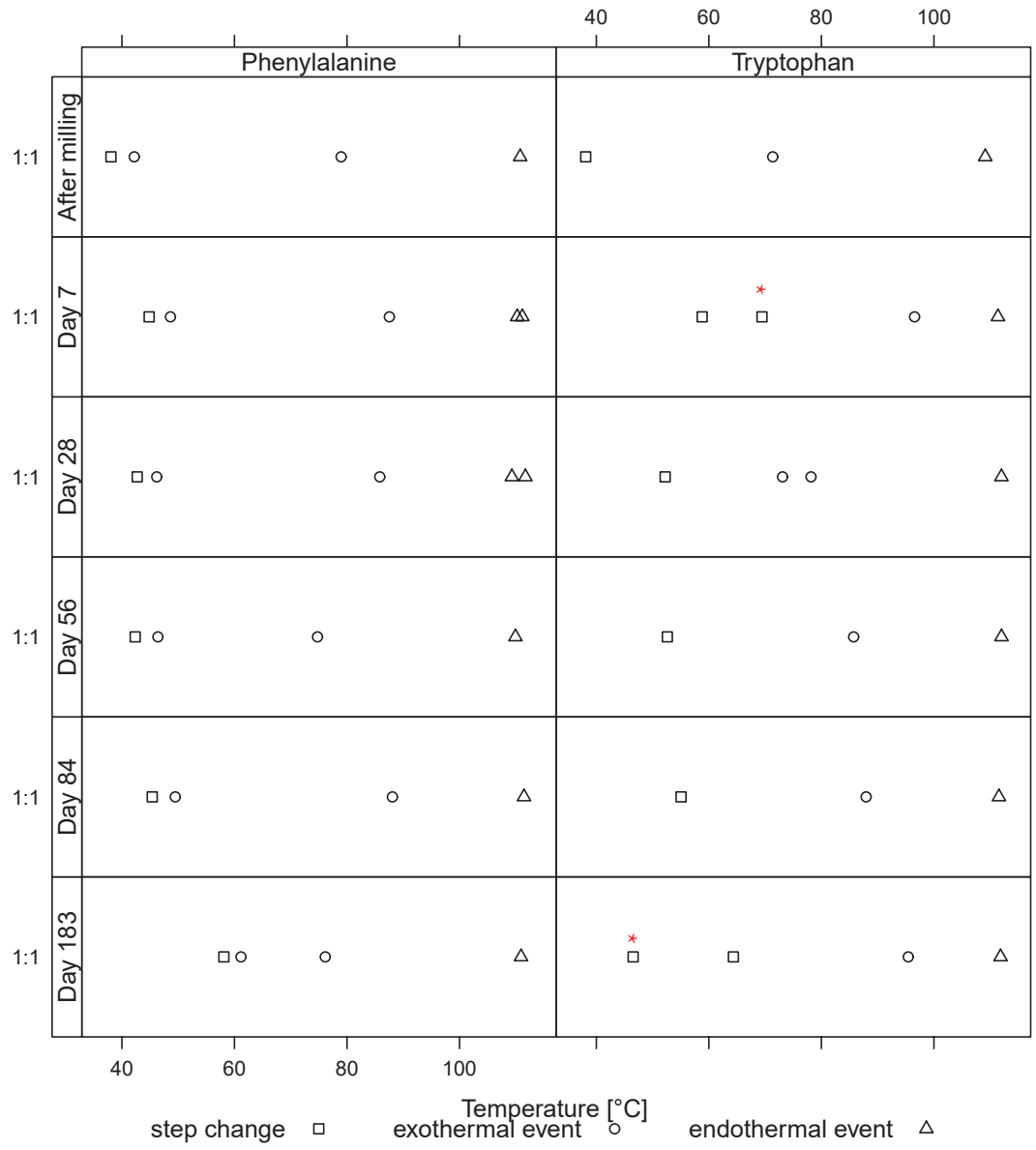


Figure 2.23. Thermal events observed on DSC-thermograms for Car-Phe and Car-Trp at 1:1 molar ratio after milling, 7, 28, 56, 84 and 183 days of storage in dry conditions (22 °C, 0% RH). The red star indicates uncertainty on the reliability of the data point.

After a comparison of the thermal behaviour of both mixtures (figure 2.23), differences and similarities were observed. In both mixtures the glass transition temperature tends to increase over time.
In specific experiments the glass transition was barely detectable and difficult to assess. When two glass transitions were potentially detected, they were systematically reported despite uncertainty on the reliability of the data, caution should be exerted during interpretation.
The T_g of the Car-Phe mixture varies slightly with the increase in T_g becoming clearly visible only after 183 days of storage. The glass transition temperature of the Car-Trp mixture is globally higher than for the Car-Phe mixture, and also tends to increase with storage time. The increase in the glass transition temperature is consistent with a partial recrystallisation of carvedilol and hence a change in the proportions between carvedilol and the amino acid of the remaining co-amorphous part.
The Car-Phe co-amorphous mixture exhibits two recrystallisation events, one immediately after the glass transition and the other one between 75 and 95 °C. The occurrence of recrystallisation immediately after the T_g and at such low temperatures is the source of physical instability for the co-amorphous mixture. The Car-Trp co-amorphous mixture displays only one recrystallisation event except after 28 days of storage. Globally, the recrystallisation temperature tends to increase with storage time. As for the Car-Phe co-amorphous mixture, the recrystallisation temperature appears correlated to the initial glass transition temperature–a higher T_g generally implying a higher recrystallisation temperature–suggesting a fixed kinetic relationship between these two events.
Endothermic events corresponding to the melting of carvedilol occur at almost always the same temperature. In the case of the Car-Phe mixture, a double melting event is sometimes visible. When a single temperature is reported, the melting signal was generally wide and asymmetric. This observation suggests that two crystalline modifications of carvedilol were present and melted within a narrow temperature range. In the case of Car-Trp only one melting event is observed which corroborates the analysis of the hygroscopicity of the different substances. It was observed that Phe is more hygroscopic than Trp and induces a partial recrystallisation of carvedilol in a hydrated from (see section 2.2.4). Despite being stored in a dry environment, water coming from the starting material and accumulated during milling was already present in the sample before storage. A small amount of water is likely still remaining which induces this recrystallisation.
The amino acid is supposed to act as a stabilising agent for the amorphous state of carvedilol. It should help to produce a co-amorphous mixture with the highest possible T_g and hinders the recrystallisation of the drug. Considering these two objectives, both Phe and Trp failed to provide the targeted stability of the co-amorphous mixture due to the recrystallisation of carvedilol over time during storage. However, if it is necessary to make a choice between the two amino acids, Trp would be the most suitable candidate since it is less hygroscopic, produces a co-amorphous mixture with a higher T_g and exhibits a recrystallisation temperature spaced out from the T_g.

Influence of the ratio on the stability of co-amorphous mixtures

The two successfully ball-milled co-amorphous mixtures Car-Phe and Car-Trp were used for the analysis of the influence of the molar ratios on their physical stability.

Therefore, mixtures with molar ratios of 3:1, 1:1 and 1:3 were stored in dry conditions and their thermal behaviour analysed using DSC. Dry storage conditions were preferred in order to remove the additional influencing factor of humidity and to be able to analyse solely the influence of the chosen molar ratio on the physical stability of these co-amorphous mixtures. DSC measurements were performed on the samples immediately after milling and after 7, 28, 56 and 84 days of storage. The observed thermal events are reported in figure 2.24.
In this section of the physical stability study, a special focus will be given to the variations in thermal behaviour over storage time. The aim is to determine whether certain molar ratios lead to a higher physical stability of co-amorphous mixtures. Regarding the differences in thermal behaviour between the ratios immediately after co-amorphisation, a previous detailed analysis is described in section 2.2.2.
According to figure 2.24, only slight variations of the thermal behaviour were observed irrespective of the molar ratios for both amino acids. The T_g varies slightly over storage although not significantly, suggesting that stability is reasonably good over time. That being said, slow recrystallisation of both carvedilol and the amino acid remains a likely scenario. This is indicated, for example in the case of Car-Trp 1:3, by the tendency of the crystallisation exotherm to decrease in intensity overtime (see appendix A.5). This suggests that a partial recrystallisation has previously occurred.
Generally, the changes observed in thermal behaviour of all the mixtures over storage time are negligible. This suggests that the carvedilol-amino acid molar ratio is not a major factor influencing the stability of co-amorphous systems.

Influence of the storage conditions on the stability of co-amorphous mixtures

The influence of the storage conditions on the physical stability of the two successfully amorphised co-amorphous mixtures Car-Phe 1:1 and Car-Trp 1:1 was assessed. This part of the study focuses on the storage conditions with a is concerned with the air humidity. As the storage temperature needs to be maintained below the T_g of the co-amorphous system in order to avoid recrystallisation, the influence of the air humidity remains to be evaluated. Therefore different humidity conditions (relative humidity, RH) were selected for the storage and are reported in table 2.3.

Table 2.3. Storage conditions for the stability test. DRY: dry conditions, CC: climate chamber, RT: room conditions and HUM: humid conditions

Storage condition	**Temperature** [°C]	**Relative humidity** [%]	**Absolute humidity** [g/m^3]
DRY	22.0 ± 2.0	0.0 ± 0.1	0.0
CC	23.0 ± 0.5	30.0 ± 5.0	6.2
RT	22.0 ± 2.0	55.0 ± 10.0	10.7
HUM	22.0 ± 2.0	99.0 ± 0.1	19.2

The milling conditions, the cold room temperature and humidity, were continuously recorded and an average value of both temperature and relative humidity is reported in table 2.4.

Table 2.4. Milling conditions.

Milling condition	**Temperature** [°C]	**Relative humidity** [%]	**Absolute humidity** [g/m^3]
MIL	5.0 ± 0.5	90.0 ± 10.0	6.1

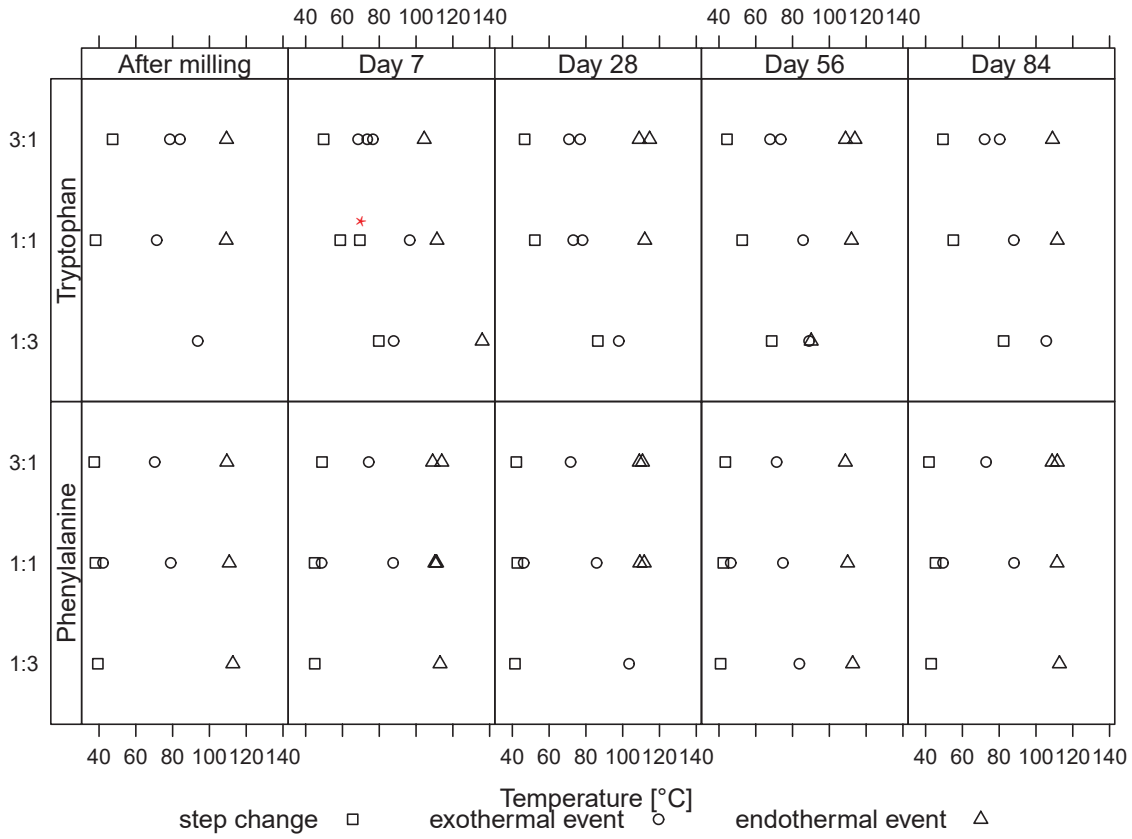


Figure 2.24. Thermal events observed on DSC-thermograms for Car-Phe and Car-Trp at 3:1, 1:1 and 1:3 molar ratios after milling and 7, 28, 56 and 84 days of storage in dry conditions (DRY, 22 °C, 0% RH). The red star indicates uncertainty on the reliability of the data point.

For both the milling conditions and the storage conditions, the absolute humidity was calculated (appendix A.7) to allow a direct comparison of the humidity conditions. The different storage conditions were then sorted from the least humid to the most humid: DRY, CC, RT and HUM. The milling conditions are close to CC conditions in terms of absolute air humidity. Among the different storage conditions, only RT had an uncontrolled relative air humidity. The relative humidity was constantly varying over time although always recorded. The relative humidity was constant in the other storage conditions with occasionally a small variation. This varying relative humidity by RT conditions might have an influence on the stability of co-amorphous systems.

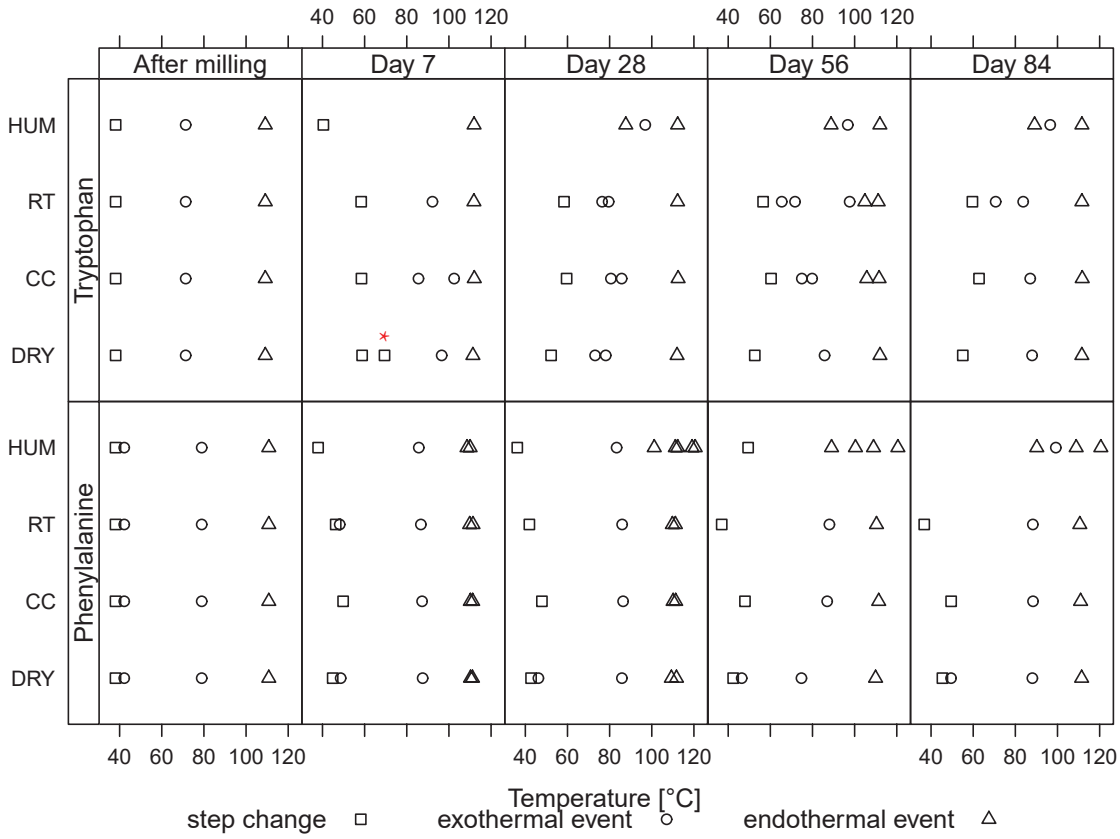


Figure 2.25. Thermal events observed on DSC thermograms for Car-Phe and Car-Trp at 1:1 molar ratio after milling and 7, 28, 56 and 84 days of storage. Storage conditions: humid conditions (HUM, 22 °C, 99% RH), room conditions (RT, 22 °C, 55% RH), climate chamber (CC, 23 °C, 30% RH), dry conditions (DRY, 22 °C, 0% RH). The red star indicates uncertainty on the reliability of the data point.

Figure 2.25 represents the different thermal events observed for Car-Phe 1:1 and Car-Trp 1:1 stored for 84 days in the different storage conditions. The thermal behaviour of mixtures stored under dry conditions exhibits the smallest variation over storage time. The T_g is slightly increased compared to after milling, which is consistent with a small loss of water after the samples were placed in dry conditions and/or with a small amount of carvedilol having recrystallised. The thermal behaviour of Car-Phe 1:1 stored under dry conditions is relatively constant over time, however small changes in the recrystallisation temperature are observed although they are considered negligible. The endothermic melting event is occasionally a doublet. This is not considered a significant difference compared to the other thermograms displaying a single melting peak, considering it was generally wide and asymmetric (see appendix A.4). This suggests that the two same crystalline modifications melt consistently within a narrow temperature range. These crystalline modifications are, according to their melting temperature, forms II and III, the latter being a hemihydrate. The presence of this hydrated crystalline form in the mixture suggests that even when stored in dry conditions, the water originating from the single substances before amorphisation and/or the water accumulated during milling can remain in the co-amorphous systems. Regarding the Car-Trp mixture stored under dry conditions,

similarly to Car-Phe, the recrystallisation signals vary over time. Nevertheless, a consistency in the thermal behaviour is still observed over storage time.
The other storage conditions appear to have a stronger influence on the stability of the Car-Phe and Car-Trp co-amorphous mixtures. The T_g of the mixtures tends to decrease as the absolute humidity in the environment increases. The decrease in T_g is also observed over time in theses conditions, particularly for the Car-Phe mixture. Moreover, the T_g disappears already after 28 days of storage in HUM conditions for the Car-Trp mixture. This change is due to water uptake in the co-amorphous mixture and the disappearance of the T_g suggests an almost complete recrystallisation of the samples.
Under HUM conditions, several endothermic events were observed especially starting from 28 days of storage. These endothermic events were not easily identifiable as one of the known polymorphs or pseudopolymorphs of carvedilol (see appendix A.3). These could be water evaporation (especially between 80 °C and 100 °C) or alternatively be the result of carvedilol or amino acid degradation products.
Globally, dry conditions for the storage of co-amorphous systems provide the most stable thermal behaviour. Humidity provokes an accelerated recrystallisation of the samples and is hence a factor of instability. The humidity conditions appear to be a key factor to control also during amorphisation.

Stability of ball-milled and freeze-dried co-amorphous mixtures

Previously, a difference was observed between ball-milled and freeze-dried Car-Asp 1:1 regarding the success of amorphisation (section 2.1) and the nature of the stabilising intermolecular interactions between carvedilol and aspartic acid (section 2.2.3). Freeze-dried Car-Asp 1:1 proved to have desirable properties towards a successful and potentially more stable co-amorphous system. To evaluate the stability of Car-Asp 1:1 BM and FD, XRPD measurements were performed directly after co-amorphisation and subsequently after approximately 25 weeks of storage under room conditions (RT, see table 2.3). Figure 2.26 represents the XRPD patterns from theses measurements. Ball-milled Car-Asp 1:1 could not be obtained as a totally co-amorphous mixture mainly due to residual crystalline aspartic acid. After approximately 25 weeks of storage under room conditions, crystalline reflection signals corresponding to carvedilol are also observed. A particular reflection peak at 5.89° suggests that carvedilol has recrystallised in form II.
After freeze drying Car-Asp 1:1, complete amorphisation was successfully accomplished. After approximately 25 weeks of storage, the sample remained totally amorphous. However, it is possible to see a difference in the shape of the halo corresponding to amorphous structure in the XRPD patterns. This difference suggests a change in the near-range structure of the co-amorphous mixture, which might also be the result of polyamorphism. Another interpretation is that a microcrystalline structure may still be present after ball milling, although below the XRPD detection level [70]. To assess this change and analyse the near-range structure of amorphous substances, a different equipment is required, for example, a synchrotron radiation equipment [79]. It is observed that the freeze-dried Car-Asp 1:1 mixture is by far more physically stable than ball-milled Car-Asp 1:1.

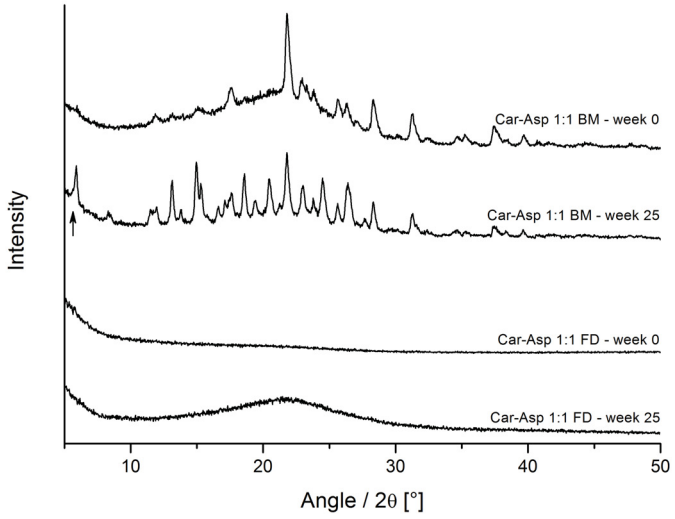


Figure 2.26. X-ray powder diffractograms of Car-Asp 1:1 freeze dried (FD) and Car-Asp 1:1 ball milled (BM) after amorphisation (week 0) and after approximately 25 weeks of storage at room conditions (RT, 22 °C, 55% RH).

In conclusion, in this stability study it was determined that the choice of amino acid has no major influence on the stability of the co-amorphous systems. Neither Phe nor Trp led to the stabilisation of the co-amorphous systems by avoiding recrystallisation. The molar ratio between carvedilol and the amino acid also did not play a significant role and no clear advantage could be identified for Car-Phe and Car-Trp mixtures. Regarding the storage conditions, humidity proved to be detrimental to the physical stability of theses co-amorphous systems. On the contrary, dry conditions offer the highest stability to the co-amorphous systems in terms of thermal behaviour over time. Finally, it was demonstrated that freeze drying provides a better stability compared to ball milling in the case of the Car-Asp 1:1 co-amorphous mixture. This suggests that the amorphisation method has a great influence on the subsequent physical stability of co-amorphous systems.

2.2.8. Summary: Overview of the characteristics of co-amorphous systems and development of a support tool for the development of co-amorphous systems with an amino acid

The characterisation of co-amorphous systems based on carvedilol main factors influencing their amorphisation susceptibility and their physical stability. The main objective of co-amorphisation is to enhance the solubility of the drug while at the same time ensuring adequate physical stability. In the case of carvedilol the intrinsic dissolution rate was increased although the equilibrium solubility was not enhanced. It was also observed that a salt formation is possible with L-aspartic acid albeit only via the freeze drying process. The co-amorphous salt exhibited the highest intrinsic dissolution rate and the best stability properties. Beside the freeze-dried Car-Asp co-amorphous mixture, two other amino acids were successfully amorphised

with carvedilol, namely L-phenylalanine and L-tryptophan. The amorphisation was successful by ball milling and only with the neutral amino acids. The stability of these mixtures was lower than for freeze-dried Car-Asp. Among the two successfully amorphised mixtures by ball milling, L-tryptophan was considered the best stabilising agent. It gives rise to the highest T_g of any mixtures. As the carvedilol-amino acid ratio does not seem to have an influence on the stability of the ball-milled mixtures, the ratio with the higher amount of L-tryptophan would be considered optimal since it provides the highest T_g. The high T_g of the mixture offers the best protection against high ambient temperatures and reduces the risk of recrystallisation. Moreover, the storage in dry conditions could potentially extend the shelf life of the mixture.

Considering all the factors which may influence the characteristics of co-amorphous systems, it was possible to develop a draft decision tool. This tool aims to guide the development of co-amorphous systems composed of a drug and an amino acid. Based on the results obtained for carvedilol a decision tree was developed (unshaded cells in figure 2.27). This decision tree includes some extrapolations especially regarding neutral drugs and acidic drugs (shaded cells in figure 2.27). These extrapolations were drawn up in a symmetric manner to the basic drug and remain a plausible scenario according to literature where research groups worked with acidic drugs such as indomethacine [10, 57, 61]. This tool could be useful for the development of co-amorphous systems when the drug has a lower T_g than the majority of the amino acids. This decision tree cannot in any circumstances be used as a rule valid in every case. It could be helpful when an extensive screening of all the amino acids at varying ratios is not considered, for example when the amount of drug is limited.
Before using this tool, it is necessary to first determine the optimal method of amorphisation. The decision tree (figure 2.27) helps to select the amino acid, the starting ratio and the storage conditions.
Figure 2.27 is centered around the acidic/basic nature of the drug being studied. The initial investigation should focus on identifying an amino acid with an opposite acidic/basic nature to the drug, in order to maximise the chances of obtaining a co-amorphous salt, which generally shows an enhanced physical stability. The drug-amino acid ratio ought to be chosen based on the chemical functional groups of each molecule that could interact together to form a salt. It is also important to keep in mind that the success of co-amorphous salt formation may depend on the amorphisation method.
When a salt cannot be obtained, the decision tree expands through a proposed set of criteria to help identify the most suitable amino acid. In this case neutral amino acids seem to be more successful than other amino acids. Note that this tool offers no guarantee that a totally co-amorphous and physically stable system will be obtained. However, the chances of success are maximised.

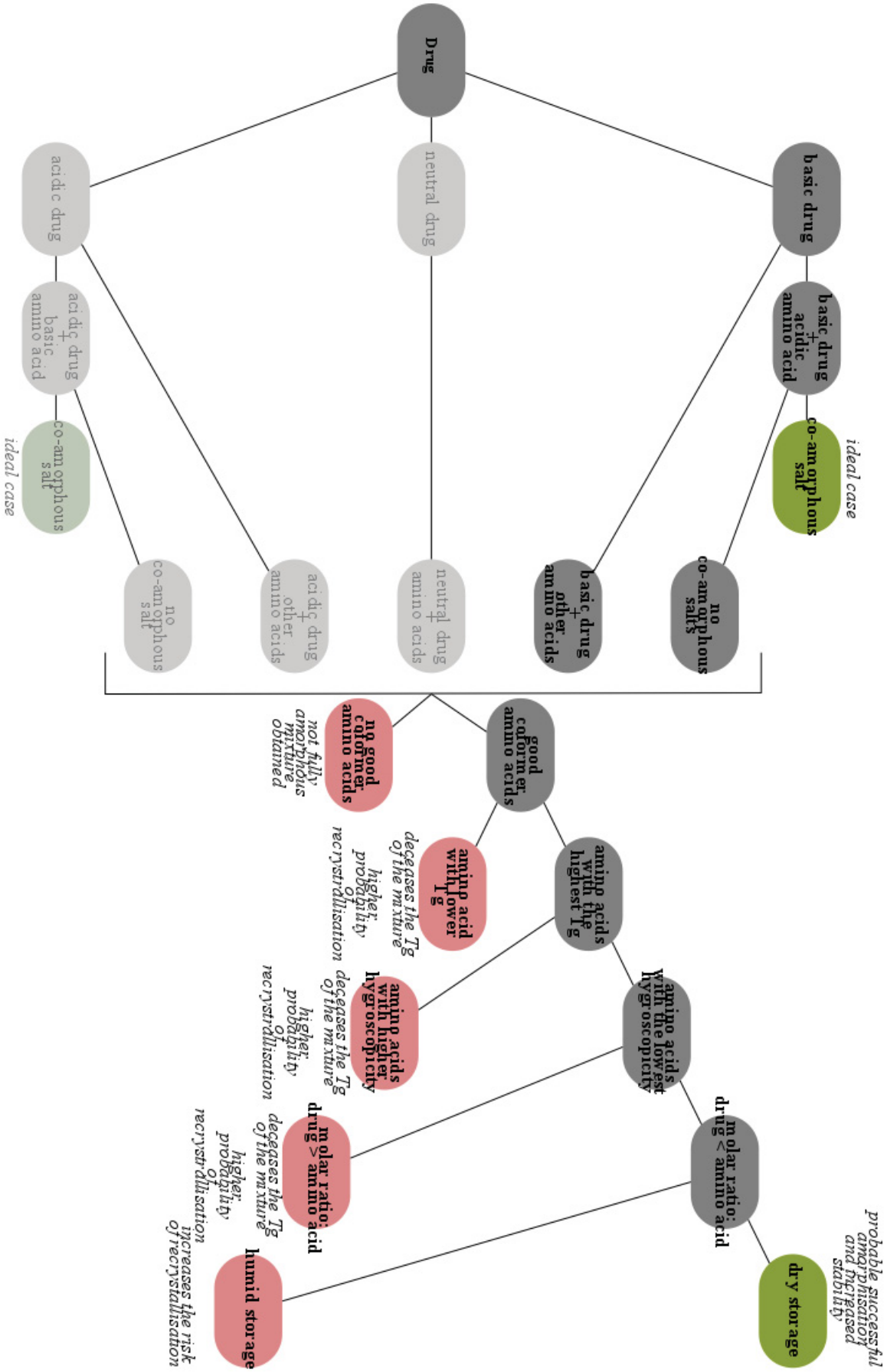


Figure 2.27. Decision tree towards the selection of the most suitable amino acid for co-amorphisation with a drug.

2.3. Dissolution profile of carvedilol tablets

Tablet formulation is one of the potential applications of co-amorphous systems. The main objective of this section is to formulate carvedilol-based tablets in order to analyse their dissolution profile. Dissolution profile analysis of tablets manufactured with co-amorphous systems is more closely related to in-vivo patient's drug dissolution data. The aim of this experiment is to test the dissolution properties of the co-amorphous systems with a standard procedure described in the European Pharmacopoeia. It is also an opportunity to determine how the presence of excipients may affect the dissolution profile of co-amorphous systems containing carvedilol.
Firstly, the manufacturing of carvedilol based tablets will be briefly described and discussed. Secondly, the dissolution profiles will be analysed.

2.3.1. Formulation of co-amorphous systems in tablets for dissolution profile

Car-Asp 1:1 was used for the manufacturing of tablets for two reasons: (1) Car-Asp 1:1 was obtained as a co-amorphous salt and in a relatively stable form, (2) even as a physical mixture (crystalline state), this pair of substances exhibited an enhanced intrinsic dissolution rate. A dosage strength of 25 mg of carvedilol was selected in line with a typical market formulation.
Freeze-dried Car-Asp 1:1 was discarded for the tablet formulation due to the non-particulate physical structure of this sample, which made the homogeneous mixing with excipients unfeasible. Consequently, ball-milled Car-Asp 1:1 was used despite being partially amorphous.
For this purpose, the ball-milled sample was made homogeneous by a slight crushing of the aggregate particles and by excluding the coarse particles. Usual excipients were selected for the formulation such as Ludipress®, Aerosil® and magnesium stearate. The proportions of each component of the formulation were adapted in order to obtain a powder mixture with sufficient flowability. Given the limited supply of ball-milled substances (max. 1 g at a time), the quality testing was restricted to key experiments and not using the required quantity of the European Pharmacopoeia tests.
A fixed amount of mixed powder was weighed for each tablet and the tablets were pressed manually. Therefore, tablets were assumed to contain a homogeneous content of carvedilol. The compacting process was performed in order to obtain tablets with a radial resistance to crushing between 10 and 20 N. These values were chosen in order to decrease the risk of recrystallisation of the amorphous samples due to pressing (the impact of pressing on recrystallisation not being evaluated) while obtaining tablets sufficiently resistant for the aimed dissolution tests. Disintegration tests were performed according to the European Pharmacopoeia to ensure that disintegration would not be influencing the dissolution study outcomes. All produced tablets were disintegrating in less than 2 min.
Using the same manufacturing process, tablets containing carvedilol either in an amorphous state or in a crystalline state, in presence of aspartic acid or not, were prepared for comparison.

2.3.2. Dissolution profile of 25 mg carvedilol tablets

The analysis of the dissolution profile of an API formulated in tablets is of great importance. In the case of carvedilol and carvedilol-based co-amorphous systems, only solubility and intrinsic dissolution tests were previously performed. The dissolution behaviour of an API may be modified in presence of excipients.
A dissolution test was hence performed in 0.1 M HCl, at a pH reflecting the gastric fluid where carvedilol is supposed to be dissolved. Tablets previously described in sections 2.3.1 and 3.2.12 were submitted to dissolution testing. Tablets composed of Car-Asp 1:1 ball milled were tested and compared to reference tablets composed of carvedilol crystalline (form II), carvedilol melt quenched (amorphous) or Car-Asp 1:1 as a physical mixtures, each containing 25 mg of carvedilol.
Figure 2.28 represents the dissolution profiles for the different types of tablets (the method is described in section 3.2.16). It is observed that all manufactured tablets allow the dissolution of at least 80 % of carvedilol within 15 minutes. Secondly, two recrystallisations of carvedilol are observed (through a sudden decrease in carvedilol concentration in solution) at time points 30 or 40 min and 120 min. These recrystallisations of carvedilol occur systematically and are not associated with a change in the pH of the dissolution medium. Interestingly, the starting physical form of carvedilol (either amorphous or crystalline) has an influence on the time point of the first recrystallisation. When carvedilol was initially in an amorphous state (carvedilol MQ or Car-Asp BM), the recrystallisation occurs after 40 min, which is 10 min later than for crystalline form II (carvedilol crystalline and Car-Asp PM). This retardation of recrystallisation could be seen as an advantage due to the longer time when carvedilol remains in solution at the beginning of the dissolution process. This time might be crucial to allow the absorption of the drug. Nevertheless, an *in vitro/in vivo* correlation remains necessary to confirm this benefit.
An identification of the recrystallised forms during the dissolution has proved not possible due to the small amount of recrystallised carvedilol (about 10 mg), diluted in a total volume of 500 ml of medium. Moreover, insoluble excipients were present in the tablets and hindered the collection of carvedilol crystals in addition to the short time frame available to collect them (max. 10 min). However, it is suspected that the crystallised carvedilol is a hydrate and crystallises when a certain concentration of carvedilol (also local concentration) is reached. When this concentration is reached crystal seeds induce the recrystallisation. Regarding the second recrystallisation, the kinetics of dissolution and recrystallisation are comparable among the dissolution profiles.

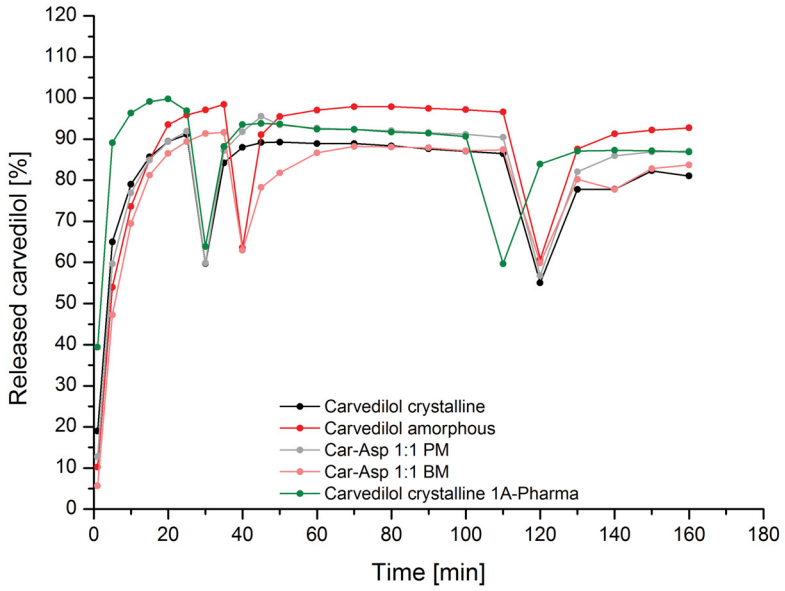


Figure 2.28. Dissolution profiles from 25-mg carvedilol tablets in 0.1 M HCl at 37 °C ($n = 6$). Tablets composed of Car-Asp 1:1 BM, Car-Asp 1:1 PM, carvedilol form II (cryst.) or carvedilol melt quenced (am.). Marketed tablet formulation: Carvedilol 1A-Pharma 25 mg.

Due to the atypical dissolution behaviour of carvedilol formulated in tablets, a comparison was performed with a marketed carvedilol tablet formulation. Tablets from 1A-Pharma containing 25 mg of carvedilol were also analysed and their dissolution profile is reported in figure 2.28. The exact composition of the marketed tablets is not known, the ingredients being reported in table 2.5 without proportions. A comparison was made between the ingredients of the manually-produced tablets and those of the marketed tablets. A higher amount of crosspovidon is however suspected in the marketed tablet based on the listing order of the ingredients in the product label and the faster release of carvedilol during the first ten minutes of the dissolution test. Both recrystallisations are also observed for the marketed formulation. Carvedilol was initially in its crystalline form and recrystallised after 30 min, similarly to the manufactured crystalline formulations (carvedilol crystalline and Car-Asp 1:1 PM). The second recrystallisation occurs however 10 min earlier than for the manually-produced tablets. This phenomenon might be linked to the slight difference in proportions of the used excipients.

Table 2.5. Composition of the tablets used for the dissolution test.

Manufactured tablets	1A-Pharma tablets
Carvedilol / Car-Asp 1:1	Carvedilol
Lactose monohydrate	Lactose monohydrate
Povidon K30	Crosspovidon
Crosspovidon	Povidon K30
Silicon dioxide	Silicon dioxide
Magnesium stearate	Magnesium stearate

2.3.3. Summary: Benefits of amorphisation on the dissolution profiles of carvedilol tablets

It appears that the recrystallisation of carvedilol is an inevitable phenomenon during the dissolution of tablets. Regardless whether carvedilol is initially in an amorphous or crystalline state, recrystallisation occurs systematically. This phenomenon will be likely the limiting factor for any attempt to enhance the solubility/dissolution rate of carvedilol by any physical method. Based on this observation, dissolution testing with the crystalline drug becomes obviously one of the first tests to perform before considering this type strategy towards solubility/dissolution rate enhancement. That being said, amorphisation brought potentially a small advantage by retarding the first recrystallisation, which in some critical cases might be helpful.

2.4. Conclusions

Over this research project many aspects related to co-amorphous systems containing amino acids were studied. The most important deliverable was to characterise the solubility of carvedilol-based co-amorphous systems. As solubility enhancement is one of the major aims of the co-amorphisation, it was here demonstrated that co-amorphisation does not necessarily enhance the solubility of a drug. It was not only observed that co-amorphisation does not enhance the solubility of carvedilol but also that carvedilol, no matter in which physical state, recrystallises in solution after dissolution from a tablet. In light of these results, co-amorphisation should be reconsidered as potentially not being a good strategy towards the solubility enhancement of certain drugs.

However, many observations are useful for the development of co-amorphous system in general. Carvedilol was selected as a drug candidate representing the BCS class II. The first stage of this project consisted in comparing a range of amorphisation methods. Freeze drying (FD), melt quenching (MQ) and ball milling (BM) were used in this research project. Each of them showed advantages and disadvantages (see section 2.1.4) which can be described in terms of three main characteristics: the *flexibility* of the method, its technical *feasibility* and the *stability* of the obtained systems. Freeze drying is the most challenging method used, as it is time consuming and technically complicated with the used equipment. Its feasibility is very low especially for large amounts. This method is suitable for many substances as long as they are soluble in water, therefore the flexibility in the choice of the stabilising agent is average in comparison to the other methods. Finally, freeze drying resulted in the most stable co-amorphous system when compared to ball milling. Melt quenching does not allow the use of amino acids due to their thermosensitivity. This method was however the easiest method since only a few minutes were necessary to obtain carvedilol in an amorphous form. Carvedilol obtained by this method was more stable than ball-milled carvedilol, which was, by contrast, never totally amorphised. Ball milling is the method with the greatest flexibility in the choice of substances because it involves a dry process. However, the samples obtained by this method exhibited the worst stability despite an average feasibility. Indeed the milling process remains time and energy consuming. These properties are graphically summarised in figure 2.29.

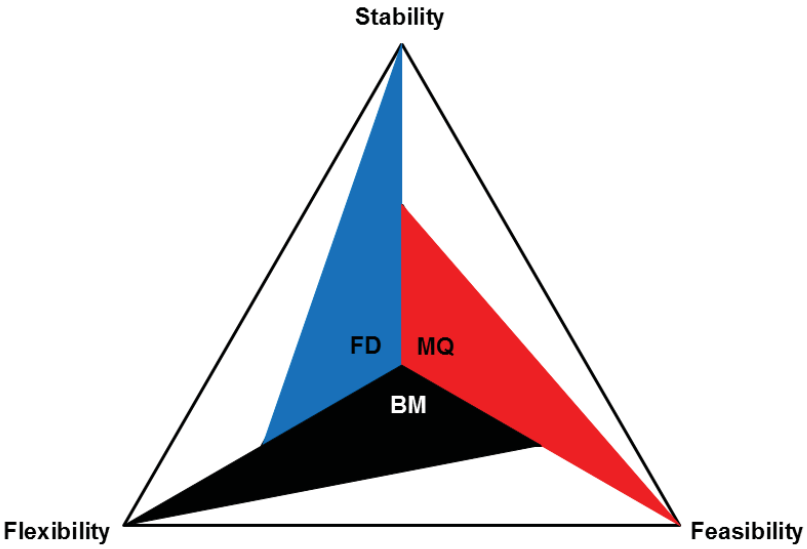


Figure 2.29. Schematic description of amorphisations methods with regard to the three main characteristics. MQ: melt quenching, FD: freeze drying, BM: ball milling.

Following the characterisation of several amino acid-based co-amorphous systems containing carvedilol, the following conclusions could be drawn:

1. The success of co-amorphisation depends on the amorphisation method. Freeze drying carvedilol with the acidic amino acid Asp was successful whereas the corresponding amorphisation by ball milling was unsuccessful. When ball milling was used, only neural amino acids gave rise to totally co-amorphous systems.
2. Analysing the thermal behaviour of co-amorphous systems provides insight into their physical stability. For example, Car-Asp 1:1 FD did not show any recrystallisation signal, and was finally the most stable sample. The measurement of the T_gs from the amorphised mixtures allows the selection of the amino acid providing the highest T_g to the mixture. A higher T_g helps to stabilise the system against high ambient temperature (during storage or from the downstream process).
3. Interactions between the drug and the amino acid could be characterised by two methods: classical NMR provides information on potential salt formation when the adequate solvent is used while FTIR provides information on weak intermolecular interactions.
4. Highly hygroscopic substances are detrimental to the physical stability of the co-amorphous system. Whenever possible the amino acid with the lowest hygroscopicity should be selected.
5. Although the solubility of carvedilol was not enhanced, the intrinsic dissolution rate was increased. Two factors seem to be important for this increase, namely the amorphous state of the system and the selected amino acid. In addition, the positive contribution of the amino acid is also observed when L-aspartic acid is combined with crystalline carvedilol as a physical mixture.
6. Neither the selected amino acid nor the Car-amino acid ratio seem to have any significant impact on the stability of co-amorphous systems in dry conditions at room temperature. By contrast, the surrounding air humidity has a great influence on the recrystallisation of the sample. Dry conditions are preferable

for the storage.

A decision tool was developed to summarise all theses points (see figure 2.27 on page 54).

When formulated in tablets, carvedilol systematically recrystallised during dissolution experiments. However, the tablets containing amorphous carvedilol exhibited a later recrystallisation in solution than for crystalline carvedilol-based tablets.

Finally, this research project contributed to extend the current knowledge on amino acid-based co-amorphous systems. Moreover, this thesis allowed the development of a methodology describing the crucial steps towards successful amino acid-based co-amorphous systems.

3. Experimental Part

3.1. Materials

The materials used are described in the following tables:

Table 3.1. Used active pharmaceutical ingredients and amino acids.

Substance	Supplier	Lot
Carvedilol	Cipla Ltd, Mumbai, India[a]	B70172
	TCI Europe N.V., Zwijndrecht, Belgium	H5FEC-HO
	TCI Europe N.V., Zwijndrecht, Belgium	U8ETN-MG
	TCI Europe N.V., Zwijndrecht, Blegium	QSPEK-MJ
L-Arginine	TCI Europe N.V., Zwijndrecht, Belgium	8XYNO-FS
L-Aspartic acid	TCI Europe N.V., Zwijndrecht, Belgium	JDQUK-MF
L-Glutamic acid	Carl Roth GmbH+Co.KG, Karlsruhe, Germany	135215097
L-Phenylalanine	TCI Europe N.V., Zwijndrecht, Belgium	RR35G-NJ
L-Tryptophan	TCI Europe N.V., Zwijndrecht, Belgium	3C8ZL-CF

[a]This carvedilol was kindly offered by the Solid State Pharmaceutics Group, Department of Pharmacy at the University of Copenhagen.

Table 3.2. Used excipients for the fabrication of Tablets.

Substance	Trade name	Supplier	Lot
Lactose, povidone, crospovidone	Ludipress®	BASF SE, Ludwigshafen, DE	69077197V0
Silicon dioxide	Aerosil®	Sigma-Aldrich Co., St. Louis, USA	081M0198V
Magnesium stearate	-	Caesar & Loretz GmbH, Hilden, DE	15305308

Table 3.3. Used substances for the preparation of buffers.

Substance	Supplier	Lot
Potassium dihydrogen phosphate	Carl Roth GmbH+Co.KG, Karlsruhe, Germany	166240108
Purified water	produced in house	diverse
Sodium hydroxide	VWR International bvba, Leuven, NL	15E260036
Chlorhydric acid	VWR International bvba, Leuven, NL	13L270504

All compounds were used as received.

3.2. Methods

3.2.1. Ball milling (BM)

1 or 2 g of pure substance or mixture of Car - amino acid were placed in a 25 ml stainless steel milling jar with 8 stainless steel balls (10 mm in diameter). The milling was

performed using the Planetary ball mill PM100 CM (Retsch GmbH, Haan, Germany) at 5 °C to 8 °C room temperature with a rotation speed of 300 rpm during 10 h to 15 h, depending on the mixture. 5-min breaks after each hour of milling were scheduled in order to allow the sample to cool down. After each 5 min break the rotation direction changed.

3.2.2. Melt quenching (MQ)

Approximately 1 g to 3 g of carvedilol were placed in an aluminium pan and melted on a hot plate at 150 °C. Immediately after melting, the pan was immersed in liquid nitrogen or placed on dry ice and maintained in position until the melt solidified. The solidified carvedilol was gently ground in a mortar in order to obtain a powder.

3.2.3. Freeze drying (FD)

500 mg of substance were dissolved in approximately 1000 ml of purified water. When lower amounts were used, the same proportions were applied. The solution was frozen with liquid nitrogen. Water of the solution was sublimated off the solution using an Alpha 2-4 LSCplus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), with a vacuum of 0.05 mbar and a shelf temperature of 20 °C.

3.2.4. Preparation of physical mixtures

Physical mixtures (PM) containing carvedilol and an amino acid were manufactured by weighing the target amount of each component. Equal volumes were initially mixed with a pestle in a porcelain mortar. Approximately equal volumes to the mixture present in the mortar were then subsequently added and mixed until an homogeneous mixture was obtained.

3.2.5. Differential scanning calorimetry (DSC)

The samples were analysed with the Differential Scanning Calorimeter Q2000 (TA Instruments, New Castle DE, USA) equipped with a RCS 90 cooler under a nitrogen gas flow of 50 ml/min, with a heating rate of 5 °C/min from 10 °C to 140 °C. Approximately 7 mg of sample were placed in a Tzero® aluminium pan and covered with a Tzero® aluminium lid (TA Instruments). The glass transition temperature was calculated as the inflection point of the glass transition with the TA Universal Analysis software (TA Instruments v.4.5A build 4.5.0.5).

3.2.6. X-ray powder diffraction (XRPD)

XRPD measurements were performed in reflection mode with an X'Pert Pro (PANalytical B.V., Almelo, The Netherlands) equipped with a Cu anode (Kα radiation λ =1.5406 Å). A current of 40 mA and an acceleration voltage of 40 kV were used and the measurements were performed from 10° to 50° 2θ with a scan step size of 0.017° and 30 s/step.
In addition, a 2D PHASER (Bruker, Billerica, MA USA) equipped with a Cu anode

(Kα radiation λ =1.541 84 Å) in reflection mode from 3° to 50° 2θ with a scan step size of 0.03° and 1 s/step was also used. A current of 10 mA and an acceleration voltage of 30 kV were used in this case.
XRPD measurements on the X'Pert Pro apparatus were performed at the Institut of Pharmaceutics and Biopharmaceutics at the Heinrich Heine University Düsseldorf.

3.2.7. Gas pycnometry

The particle density of powders was measured using an AccuPyc II 1340 gas pycnometer (Micromeritics Instrument Corp., Norcross, GA USA). A sample chamber with a capacity of 1.0 cm^3 was used and a calibration was performed before the experiments, with the corresponding ball standard. The sample cup was filled two thirds full with sample material weighed with an ABT 100-5M scale (Kern & Sohn GmbH, Balingen, Germany; d =0.01 mg). The system containing the sample was initially purged 10 times with helium. Thereafter, the volume of each sample was measured 10 times at 23.0 ± 1.0 °C with an equilibrium pressure rate of 0.005 psig/min. The particle density was calculated as the average of these 10 measurements and divided by the mass of the sample.

3.2.8. Fourier-transform infrared spectroscopy (FTIR)

FTIR measurements were performed on co-amorphous mixtures to identify the type of interaction between carvedilol and the amino acid. These were performed with an Alpha FTIR spectrometer (Bruker, Billerica, MA USA), equipped with a Platinum ATR single reflection diamond module. The powders were measured as such between 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}. A background spectrum was previously measured and subtracted from the measured sample spectra. The resulting and analysed spectra are an average of 24 spectra. The OPUS software (v.7.5, Build:7,5,18; Bruker) was used for data acquisition and processing.

3.2.9. Nuclear magnetic resonance (NMR)

^{1}H NMR was performed with an Avance HD 400 (Bruker, Billerica, MA USA) equipped with a 400 MHz magnet. A spatula tip of powder was dissolved in approximatly 600 µl of deuterated dimethyl sulfoxide and transferred in an NMR tube for measurement.
The NMR measurements were performed at the TH Köln, Faculty of Applied Natural Sciences by Mr. Alexander Kempa.

3.2.10. Dynamic vapour sorption analysis (DVS)

DVS anaylsis was performed on the crystalline materials with a SPS11 (ProUmid, Ulm, Germany). 600 mg to 800 mg of powder were placed in a previously tared aluminium pan. The temperature was maintained at 25 °C, the relative humidity was increased from 0 % to 90 % by 10 % steps and subsequently reduced to 0 % by 10 % steps. The relative humidity level was maintained constant for at least 60 min and until the mass variation was ≤0.01 % for 30 min before progressing to the next relative humidity level. The maximum cycle time was limited to 72 h and the samples were weighted at 10 min intervals.

DVS experiments were performed at the Institute of Pharmaceutics and Biopharmaceutics at the Heinrich Heine University Düsseldorf with the help of Dr. Klaus Knop.

3.2.11. Stability test

Stability tests were performed on selected amorphised samples. Directly after amorphisation the samples were split in four parts and placed into brown glass vials. These vials were stored slightly unscrewed in different humidity conditions:

- in dry conditions (DRY)—in a desiccator at room temperature,
- in stable conditions of humidity and temperature (CC)—in a climate chamber (HPP110, Memmert, Schwabach, Germany) at 30.0 ± 5.0 % RH and 23.0 ± 0.5 °C,
- in room conditions (RT)—uncontrolled room temperature and humidity,
- in humid conditions (HUM)—in a desiccator with water at room temperature.

In order to compare the humidity values independently from the temperature, the absolute humidity was calculated (appendix A.7) and is reported in table 3.4.

Table 3.4. Storage conditions for the stability test. DRY: dry conditions, CC: climate chamber, RT: room conditions and HUM: humid conditions

Storage condition	**Temperature** [°C]	**Relative humidity** [%]	**Absolute humidity** [g/m^3]
DRY	22.0 ± 2.0	0.0 ± 0.1	0
CC	23.0 ± 0.5	30.0 ± 5.0	6.2
RT	22.0 ± 2.0	55.0 ± 10.0	10.7
HUM	22.0 ± 2.0	99.0 ± 0.1	19.2

In the climate chamber condition the temperature and humidity values were controlled. For the other conditions, the temperature was not controlled, therefore an approximate uncertainty is reported in table 3.4. DSC and XRPD measurements were performed directly after ball milling and subsequently after 7, 28, 56, 84, and 183 days of storage.

3.2.12. Manufacturing of 25 mg carvedilol tablets

Tablets containing 25 mg of carvedilol were produced for dissolution tests. Carvedilol crystalline, carvedilol melt quenched, carvedilol-aspartic acid 1:1 molar ratio (Car-Asp 1:1) as a physical mixture (PM) and as a co-amorphous mixture (COA) were used for the manufacturing of the tablets. Table 3.5 presents the composition of the tablets formulations.

Table 3.5. Composition of the different 25 mg carvedilol manufactured tablets. Proportions in mass %.

Substance	Car	Car MQ	Car-Asp PM	Car-Asp COA
Car cryst.	12.5	-	-	-
Car MQ	-	12.5	-	-
Car-Asp 1:1 PM	-	-	16.59	-
Car-Asp 1:1 BM	-	-	-	16.59
Ludipress®	80.5	80.5	76.41	76.41
Aerosil®	5	5	5	5
Magnesium stearate	2	2	2	2

Mixing

The weighed powders (10 g per formulation) were placed in a bottle, which itself was placed in the cube mixer. This was done because of the small volume of the powders. For this purpose a universal motor drive (UAM 400, Pharmag, Klipphausen, Germany) equipped with a stainless steel UGD cube mixer (Pharmag) was used. The powders were mixed 15 min at a motor rotation speed of 200 rpm (equivalent to 20 rpm for the cube rotations).

Tabletting

The tabletting process was performed manually due to the small volume of mixed powders. A single punch tablet press, XP1 (Korsch, Berlin, Germany) equipped with round 9 mm in diameter punches for standard biconvex tablets was used. 200 mg of powder was weighed for each pressed tablet. The upper punch position was set in order to obtain tablets with a low radial crushing strength, approximately 20 N.

Radial resistance to crushing

The radial resistance to crushing of the manufactured tablets was measured with a PTB 302 device (Pharmatest, Hainburg, Germany). To account for the small batch size, the resistance to crushing was measured on 5 tablets for each batch.

Disintegration testing

The disintegration time was measured with a PTZ Auto 1 semi-automated device (Pharmatest, Hainburg, Germany), in purified water at 37 °C, n=3.

3.2.13. Ultraviolet-visible spectrophotometry (UV)

UV-photospectrometry was used for the quantification of carvedilol. Therefore a calibration curve was done (A.6) using carvedilol solutions in concentrations from 0.002 12 mg/ml to 0.05 mg/ml in 0.1 mol/l HCl. These solutions were measured against a blank of 0.1 mol/l HCl at a wavelength of 285 nm, with Brand® UV-Cuvettes semi-micro (Brand GmbH + Co KG, Wertheim, Germany). The measurements were

performed using a UV-1600PC Spectrophotometer (VWR International bvba, Leuven, Belgium).
UV measurements on samples were performed under the same conditions as the calibration. However, different blanks were used depending on the mixtures.

3.2.14. Solubility tests by shake flask method

Solubility measurements were performed in 25 ml of phosphate buffer pH 7.4 and in 25 ml of 0.1 mol/l hydrochloric acid. The concentration of carvedilol needed for obtaining a supersaturated solution was estimated at 180 µg/ml. Therefore, depending on the mixtures (Car-AA), the mass of mixture for 25 ml of solution was calculated. A corresponding blank containing only the amino acid in phosphate buffer pH 7.4 or 0.1 mol/l HCl was also measured in the same conditions as for the mixtures. The solutions were placed in a Titramax 1000 shaker (Heidolph, Schwalbach, Germany) equipped with an incubator (Inkubator 1000, Heidolph, Schwalbach, Germany). The solutions were shaken at 300 rpm, 72 h at 37 °C. Thereafter, the solutions were filtered using a syringe filter composed of cellulose mixed ester based membrane (cellulose nitrate and cellulose acetate) and of a 0.22 µm pore size. Eventually, they were measured by UV spectrophotometry at 285 nm using a UV-1600PC Spectrophotometer (VWR International bvba, Leuven, Belgium) and Brand UV-Cuvettes semi-micro (Brand GmbH + Co KG, Wertheim, Germany).

3.2.15. Intrinsic dissolution tests

Intrinsic dissolution tests were performed in a dissolution tester DT 626, USP 1, 2, 5 and 6 (Erweka GmbH, Heusenstamm, Germany). Approximately 150 mg of sample were placed in the die of the intrinsic dissolution apparatus. The powder was pressed 30 s with a force of approximately 9 kN (10 Ton hydraulic shop press, Baileigh industrial GmbH, Fellbach, Germany). The resulting surface of the compacted powder in contact with the dissolution media was 50.27 mm^2. The dissolution study was performed in sink conditions, in 350 ml of different medium: 0.1 mol/l HCl (pH 1), phosphate buffer at pH 4.5 and pH 7.4 at 37 °C. A stirring speed of 90 rpm was used and 1 ml aliquots were withdrawn at determined times and immediately replaced with 1 ml of buffer. The samples were analysed by UV spectrophotometry at λ =285 nm. All dissolution experiments were conducted in triplicate.
Intrinsic dissolution tests were partially performed by Mrs. Mariyam Radi and Mr. Martin Jurczyk, Faculty of Applied Natural Sciences, TH Köln.

3.2.16. Dissolution from tablets

Dissolution tests were performed on tablets containing 25 mg of carvedilol in a dissolution tester DT 626, USP 1, 2, 5 and 6 (Erweka GmbH, Heusenstamm, Germany). The dissolution study was performed in sink conditions in 500 ml of 0.1 mol/l HCl (pH 1) at 37 °C. A 90 rpm stirring speed was used and 4 ml aliquots were withdrawn at determined times and immediately replaced with 4 ml of buffer. The samples were filtered with a syringe filter composed of cellulose mixed ester based membrane (cellulose nitrate and cellulose acetate) with a pore size of 22 µm. The samples were analysed by UV spectrophotometry at λ =285 nm. All dissolution experiments were

conducted on 6 tablets.

Dissolution tests from tablet were partially performed by Mrs. Mariyam Radi, Faculty of Applied Natural Sciences, TH Köln.

A. Appendices

A.1. X-ray powder diffractograms of starting materials

X-ray powder diffractograms of crystalline substances were obtained in the same conditions as the co-amorphous mixtures and used as a reference for the assessment of the other XRPD patterns. The figures A.1.1 to A.1.6 show the diffractograms of Carvedilol crystalline, Arg crystalline, Phe crystalline, Trp crystalline, Glu crystalline and Asp crystalline.

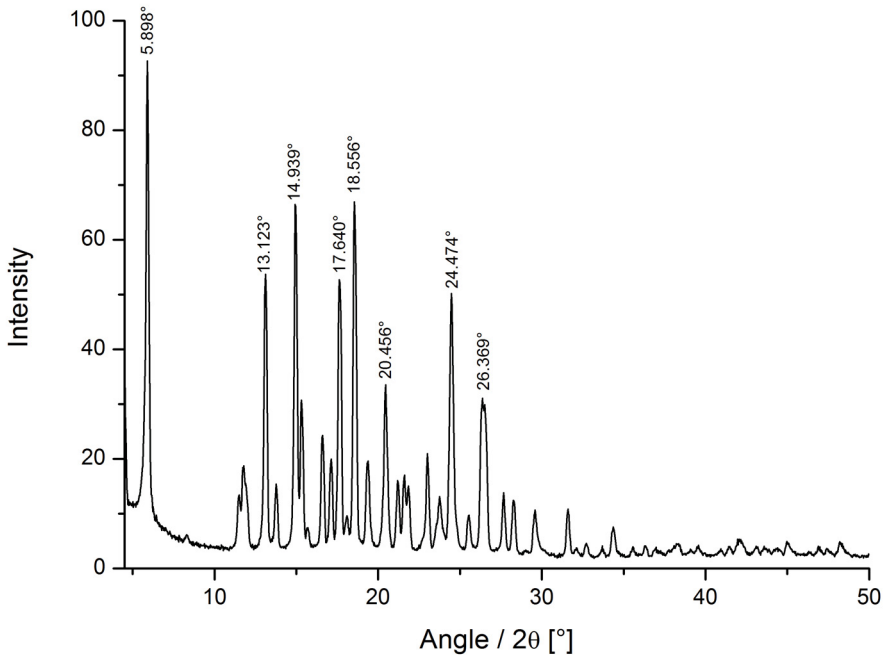


Figure A.1.1. XRPD pattern of carvedilol crystalline.

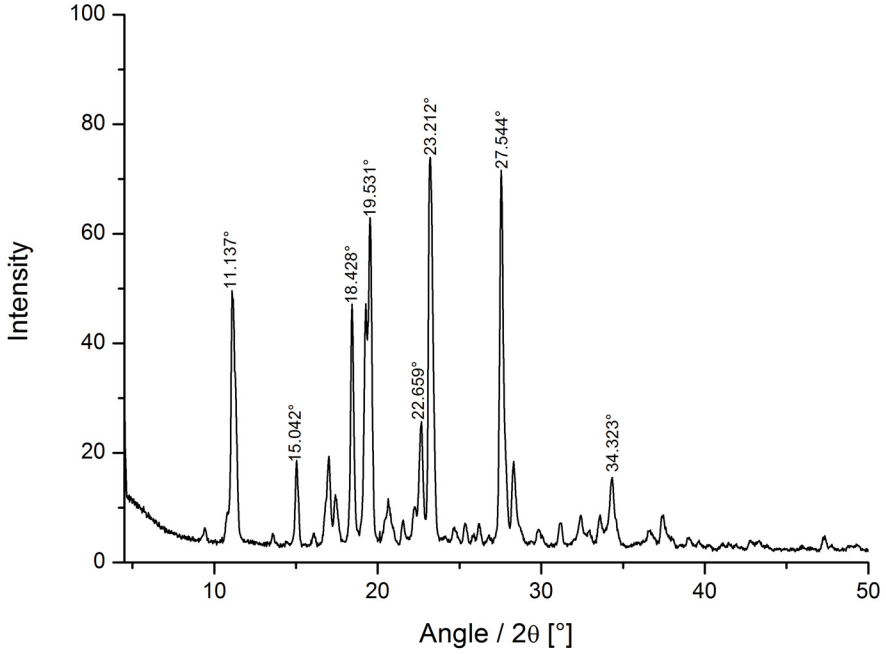


Figure A.1.2. XRPD pattern of L-arginine crystalline.

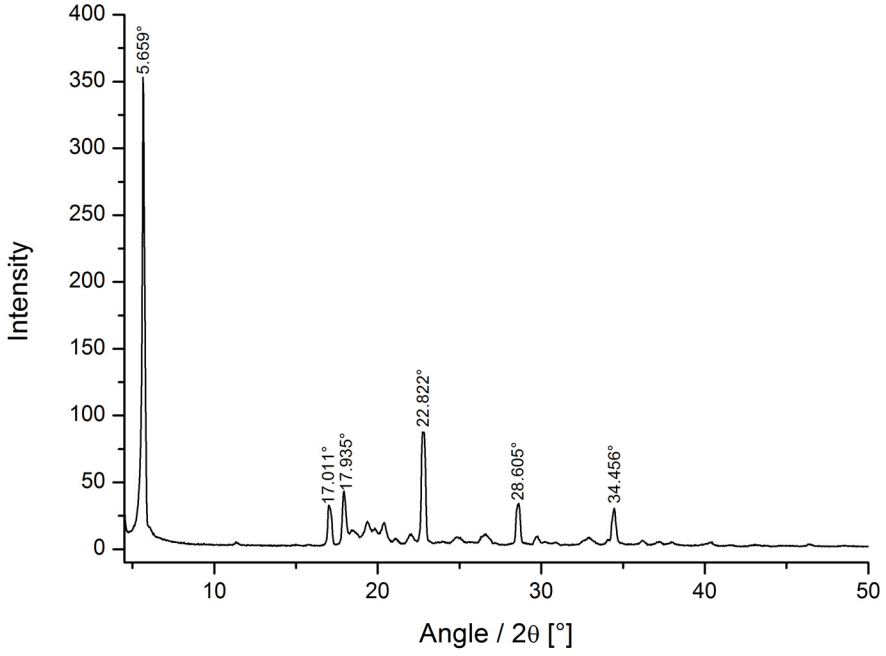


Figure A.1.3. XRPD pattern of L-phenylalanine crystalline.

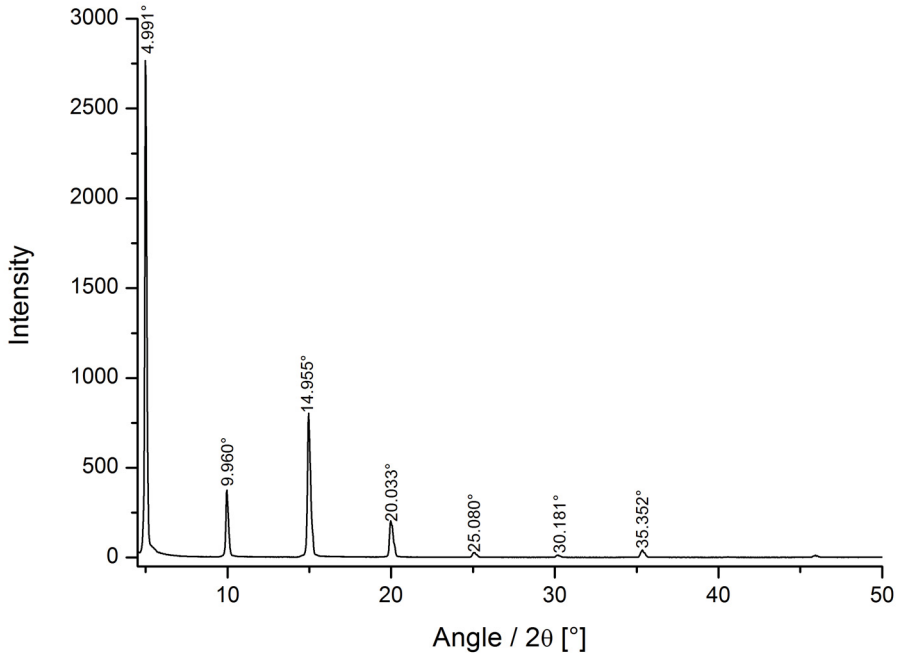


Figure A.1.4. XRPD pattern of L-tryptophan crystalline.

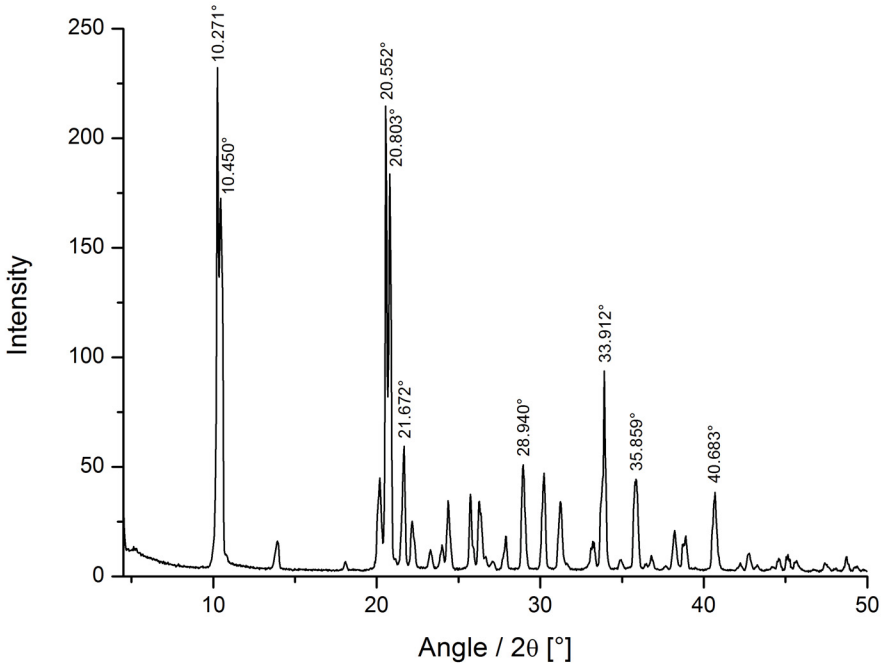


Figure A.1.5. XRPD pattern of L-glutamic acid crystalline.

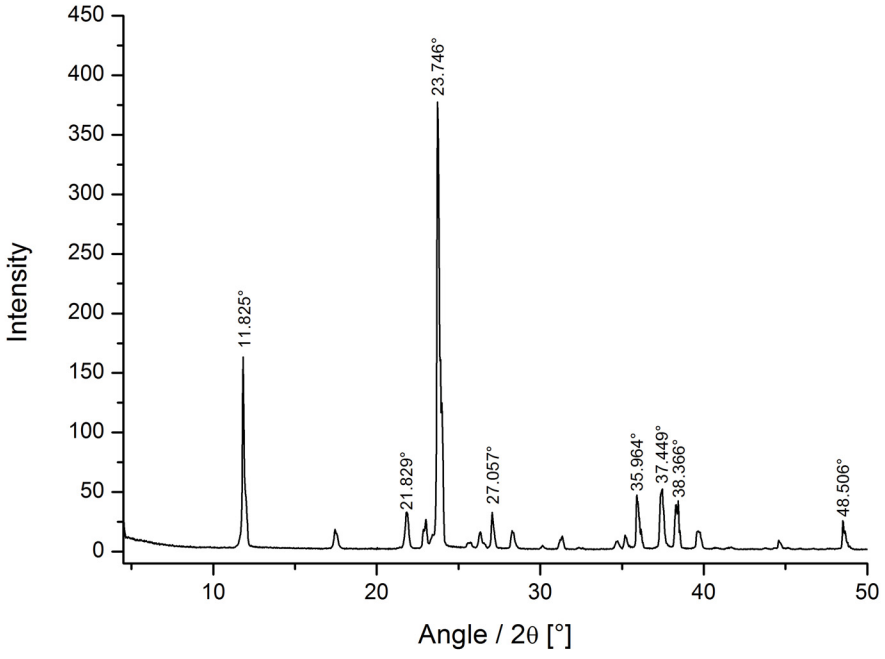


Figure A.1.6. XRPD pattern of L-aspartic acid crystalline.

A.2. Thermograms of amorphous single substances

Single substances were either ball milled or melt quenched (carvedilol) to determine their T_g. Therefore, DSC measurements were performed immediately after milling or melt quenching, even in cases where the substances were not totally amorphous. Generally the T_g is detectable, albeit for Asp for which no T_g was detected.
Figures A.2.1 to A.2.6 show the thermograms of Carvedilol MQ, Arg BM, Phe BM, Trp BM, Glu BM and Asp BM.
Temperatures of the main thermal events are indicated on the thermograms.

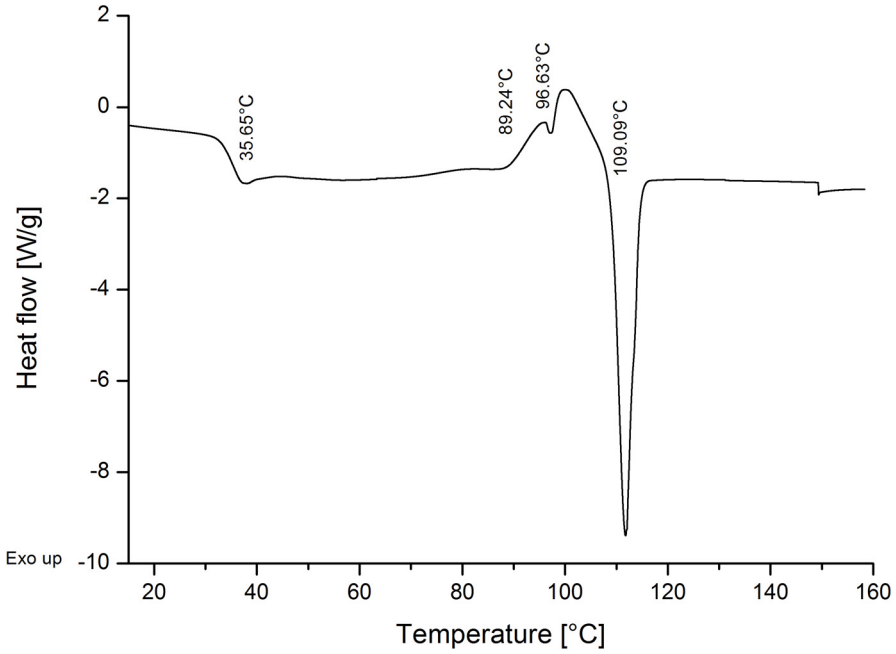


Figure A.2.1. DSC thermogram of melt-quenched carvedilol.

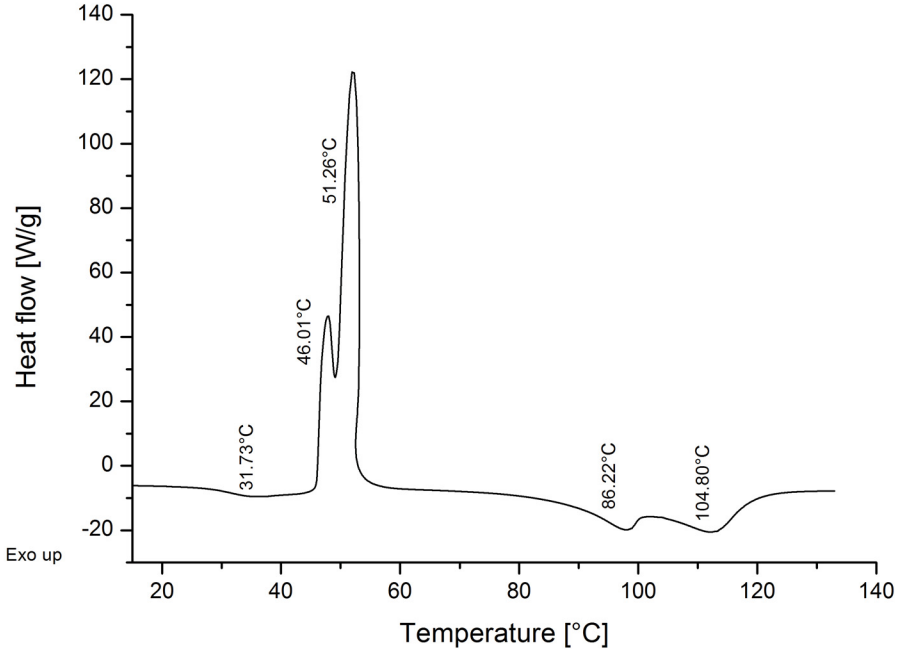


Figure A.2.2. DSC thermogram of ball-milled L-arginine.

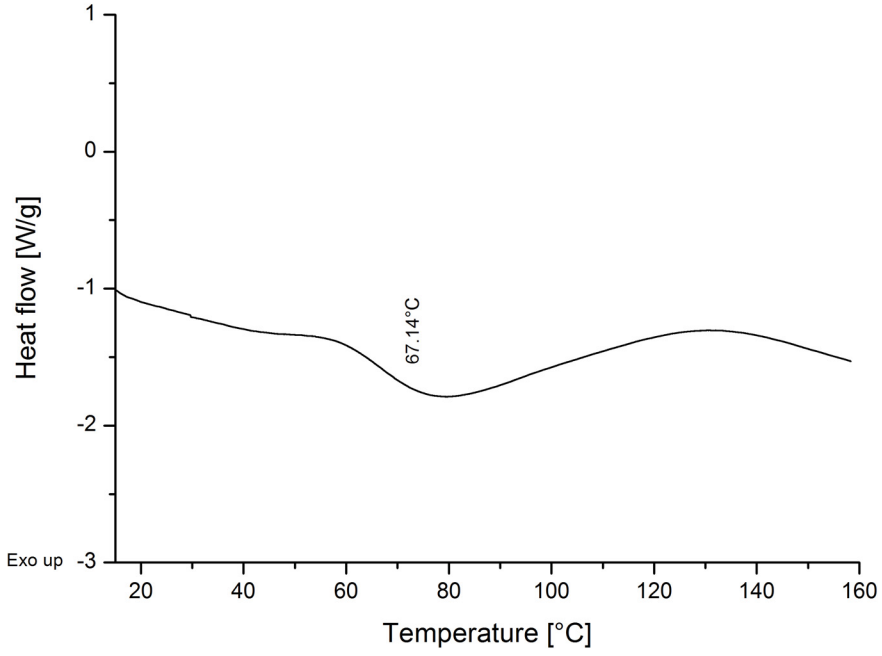


Figure A.2.3. DSC thermogram of ball-milled L-phenylalanine.

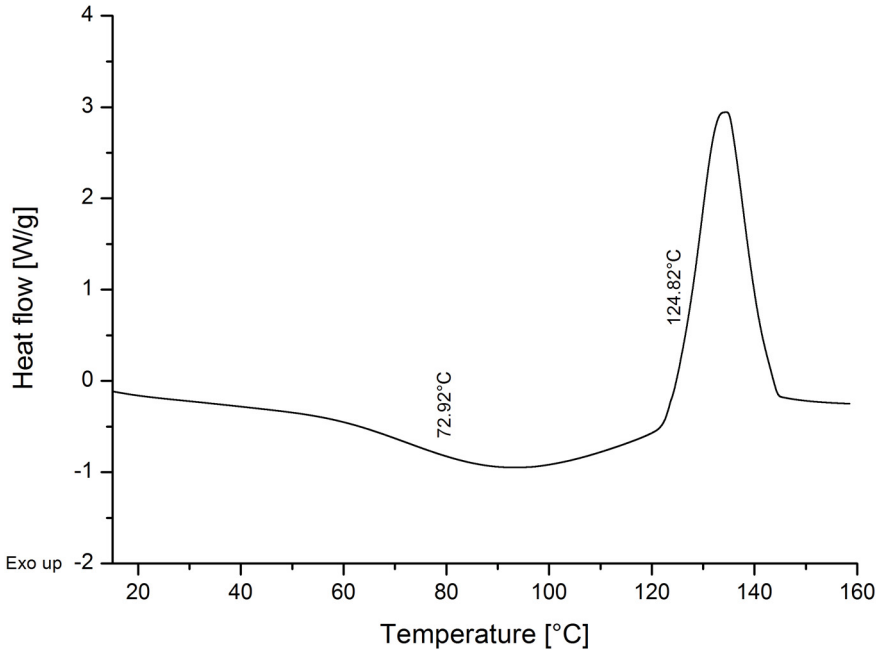


Figure A.2.4. DSC thermogram of ball-milled L-tryptophan.

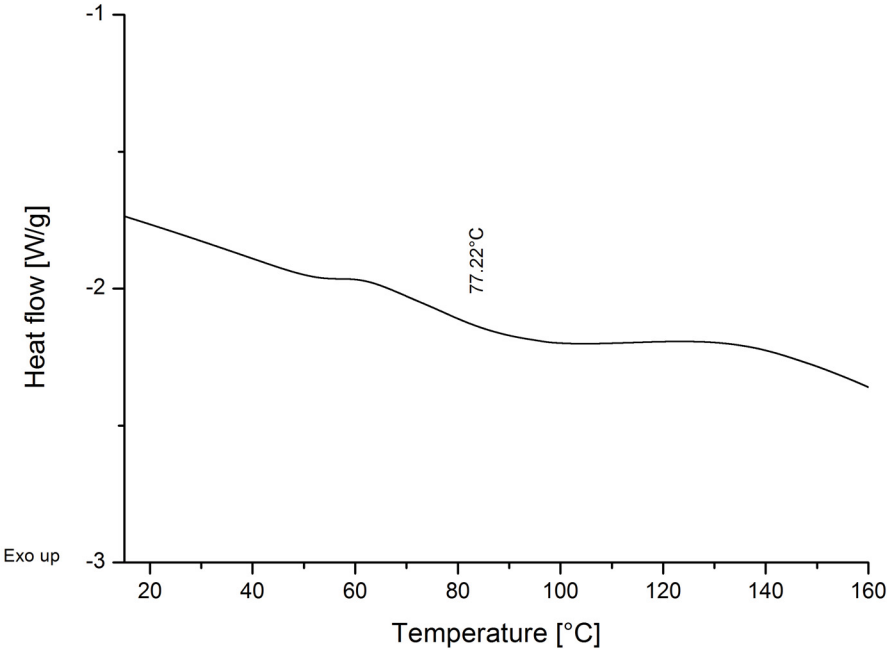


Figure A.2.5. DSC thermogram of ball-milled L-glutamic acid.

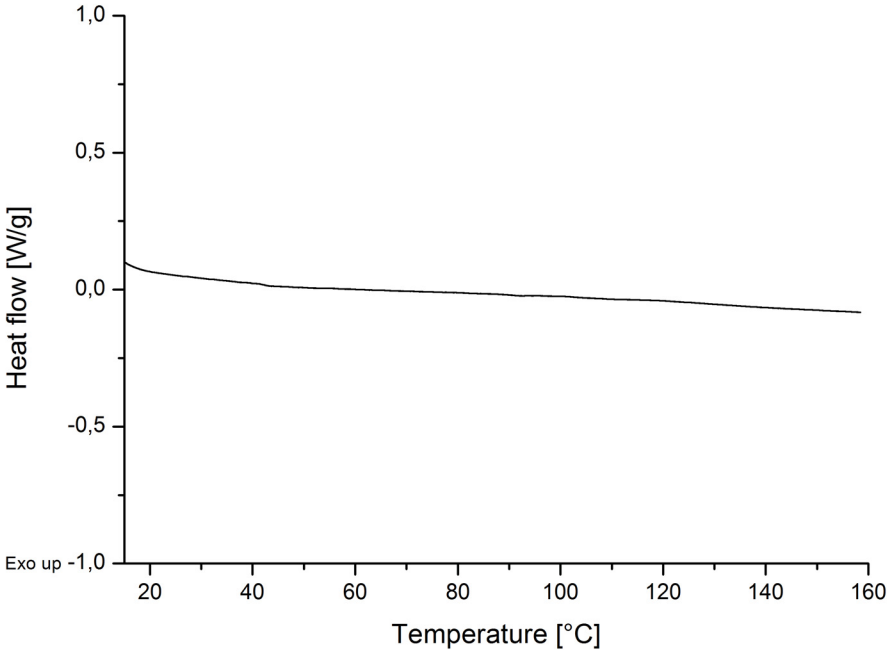


Figure A.2.6. DSC thermogram of ball-milled L-aspartic acid.

A.3. Polymorphs and pseudopolymorphs of carvedilol

This appendix provides a list of known polymorphs and pseudopolymorphs of carvedilol together with their associated properties (A.3.1).
In table A.3.2, a non-exhaustive list of polymorphs and pseudopolymorphs of carvedilol phosphate salts is presented.
More polymorphs and solvatomorphs of carvedilol also exist however not all of them are stable and making their use in pharmaceutical formulations.

Table A.3.1. Carvedilol polymorphs and pseudopolymorphs.

Form	Description	Patent	COD ID [a]	Characteristic XRPD peaks (2θ) [°]	Mp [°C]	Reference
I	Carvedilol	WO/1999/05105	7214235	9.5 - 10.8 - 12.0 - 14.5 - 19.6 - 21.5 - 22.3	123-126	[82]
II	Carvedilol	WO/1999/05105	2212191	5.9 - 14.9 17.6 - 18.5 - 24.4	114-115	[82, 83]
III	Carvedilol hemihydrate	WO/2002/00216	2018134	8.4 - 17.4 - 22.0	100 (96-110)	[76, 84]
IV	Carvedilol hydrate	WO/2002/00216	/	11.9 - 14.2 - 18.3 - 19.2 - 21.7 - 24.2	104	[76]
V	Carvedilol methyl ethyl ketone monosolvate	WO/2002/00216	/	4.1 - 10.3 - 10.7 - 11.5	desorption, recrystallisation in form II	[76]
VI	Carvedilol ethyl acetate disolvate	WO/2003/059807	/	6.5 - 7.3 - 16.0 - 30.5	74	[85]

[a] identification number of the Crystallography Open Database [80, 81]

Table A.3.2. Carvedilol phosphat salts polymorphs and pseudopolymorphs.

Form	Description	Patent	COD ID [a]	Reference
I	Carvedilol dihydrogen phosphate hemihydrate	WO/2004/002419	2222802	[86, 87]
II	Carvedilol dihydrogen phosphate dihydrate	WO/2004/002419	/	[86]
III	Carvedilol dihydrogen phosphate methanol solvate	WO/2004/002419	/	[86]
IV	Carvedilol dihydrogen phosphate dihydrate	WO/2004/002419	/	[86]
V	Carvedilol dihydrogen phosphate	WO/2004/002419	/	[86]
VI	Carvedilol hydrogen phosphate	WO/2004/002419	/	[86]

[a] identification number of the Crystallography Open Database [80, 81]

A.4. DSC thermograms of the stability study: Car-Phe

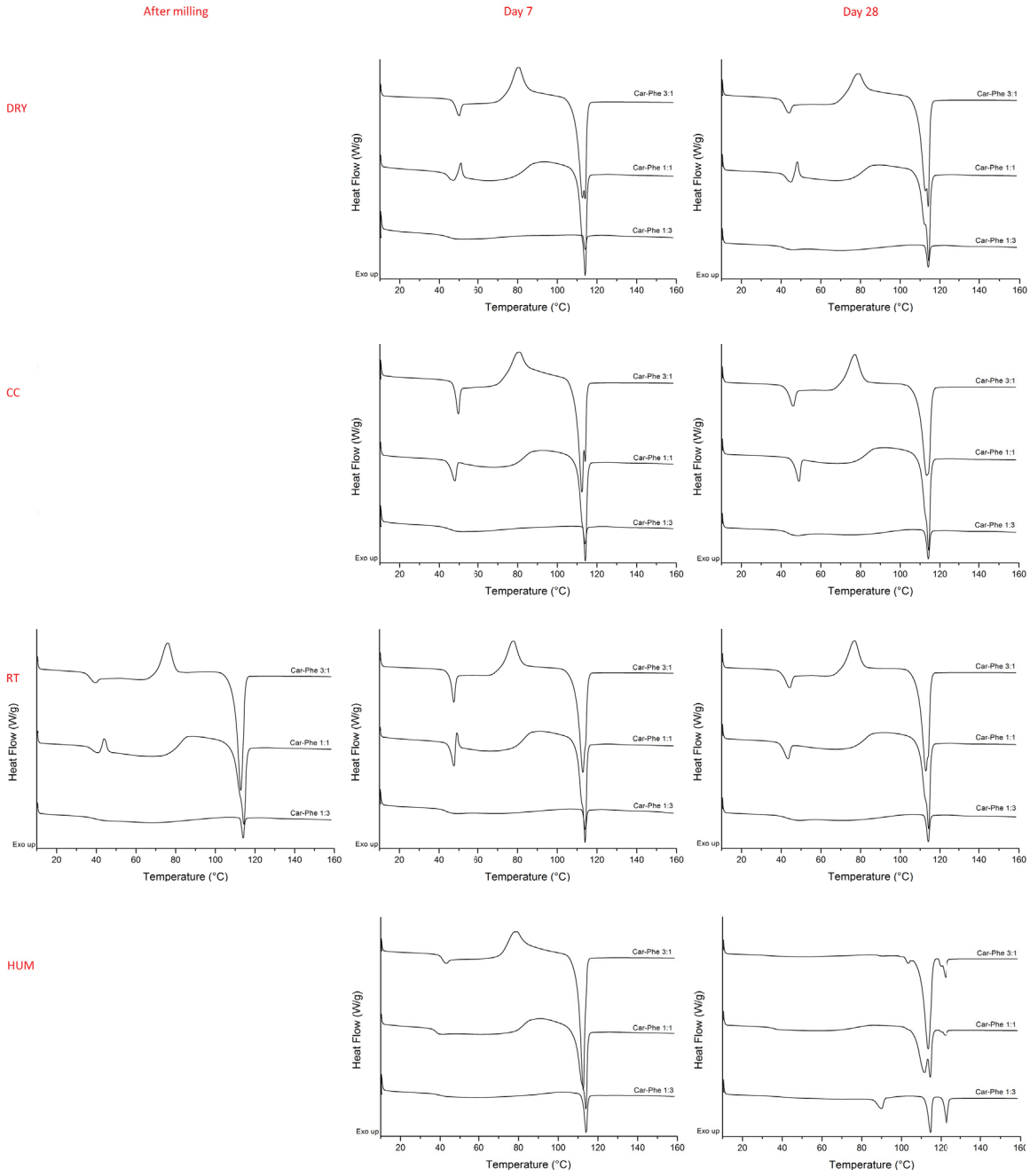
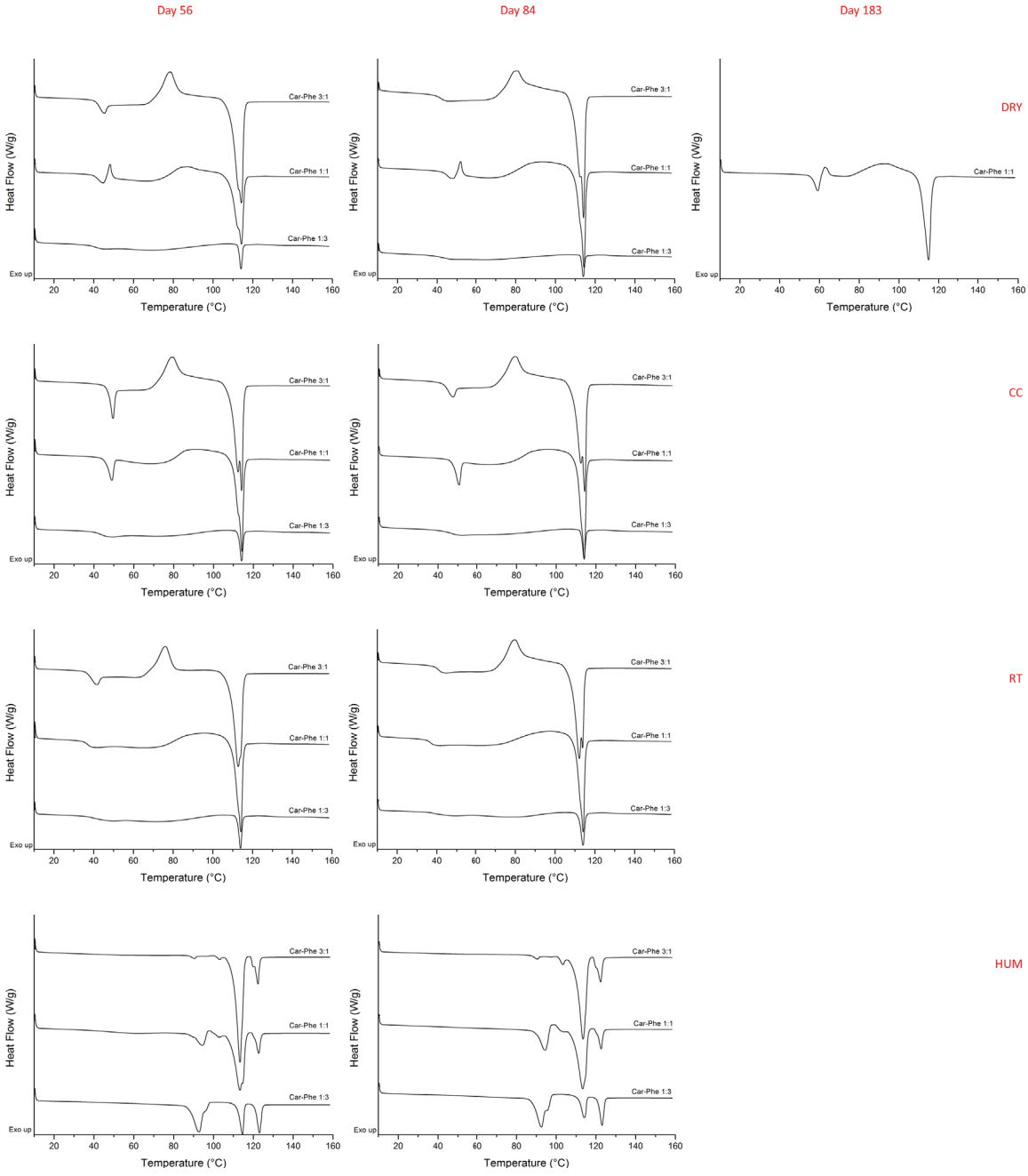


Figure A.4.1. DSC thermograms of ball-milled Car-Phe co-amorphous mixtures at different molar ratios and stored in different conditions over 183 days.


Day 56
Day 84
Day 183
DRY
CC
RT
HUM
Car-Phe 3:1
Car-Phe 1:1
Car-Phe 1:3
Heat Flow (W/g)
Exo up
Temperature (°C)
20
40
60
80
100
120
140
160

A.5. DSC thermograms of the stability study: Car-Trp

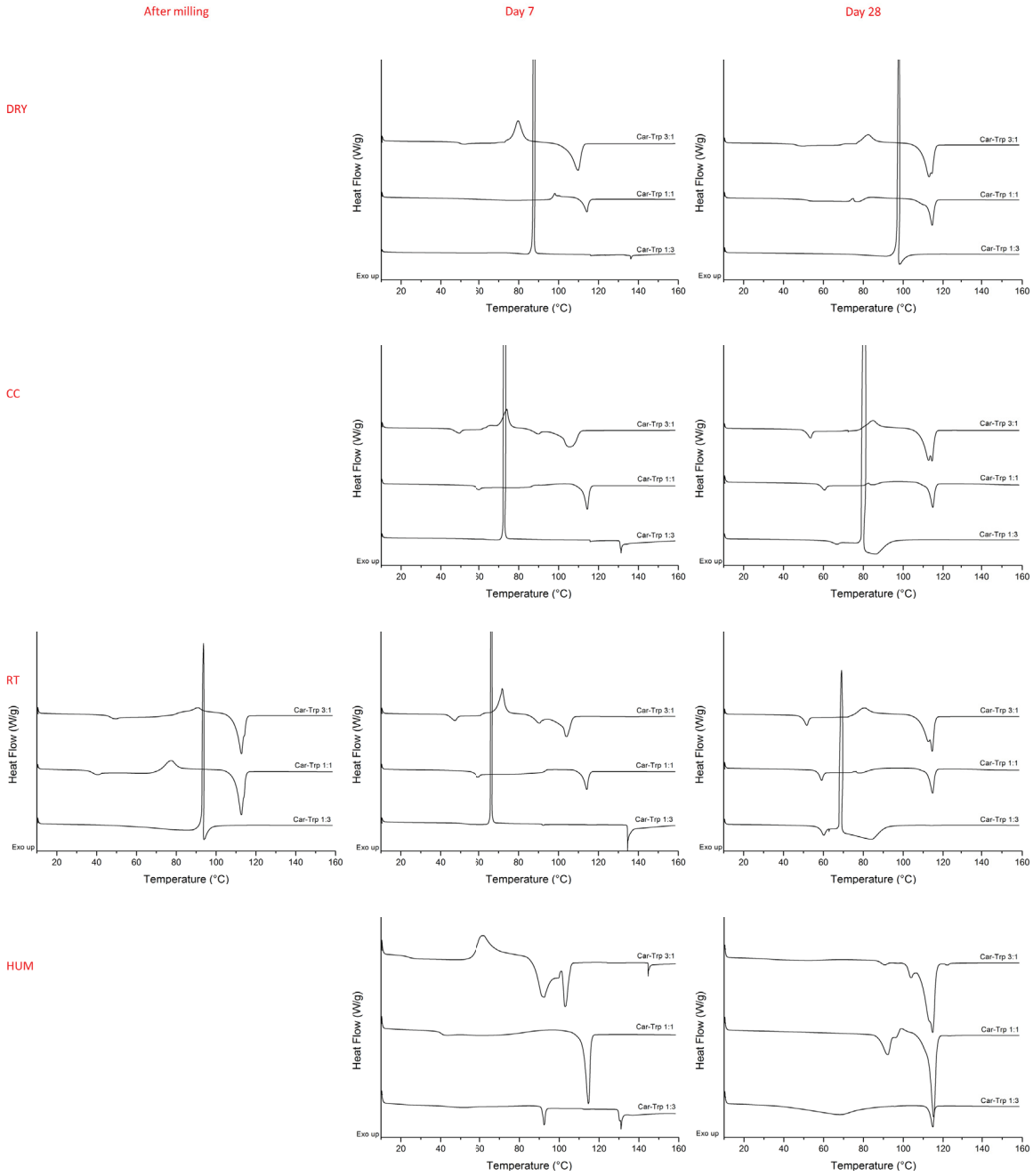
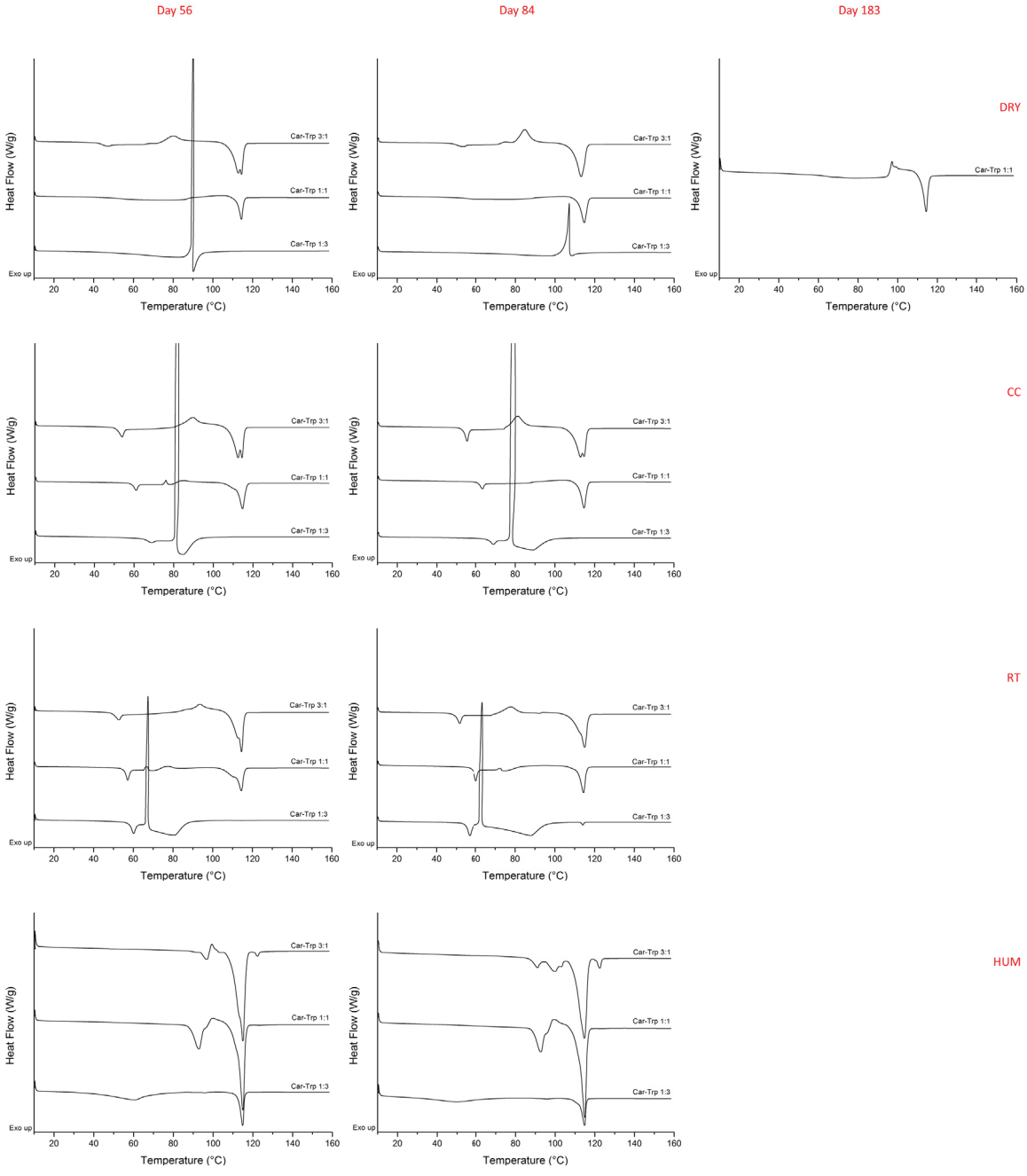


Figure A.5.1. DSC thermograms of ball-milled Car-Trp co-amorphous mixtures at different molar ratios and stored in different conditions over 183 days.


Day 56
Day 84
Day 183
DRY
CC
RT
HUM
Heat Flow (W/g)
Exo up
Temperature (°C)
Car-Trp 3:1
Car-Trp 1:1
Car-Trp 1:3

A.6. UV-photospectrometry: Calibration curve

Different solutions of carvedilol were prepared in 0.1 M HCl with concentrations between 0.05 mg/ml and 0.002 12 mg/ml. The absorption was measured at a wavelength of 285 nm. A solution of 0.1 M HCl was used as a blank. Table A.6.1 represents the measured absorption values for each solution.

Table A.6.1. Solutions of Carvedilol used for the calibration curve and their UV absorption at 285 nm

Concentration [mg/ml]	Absorption
0.05	1.164
0.045	1.034
0.0405	0.919
0.0364	0.823
0.0328	0.737
0.0295	0.653
0.0265	0.583
0.0238	0.519
0.0214	0.465
0.0192	0.418
0.0173	0.372
0.0156	0.339
0.014	0.302
0.0105	0.227
0.005 25	0.114
0.002 62	0.067
0.002 36	0.060
0.002 12	0.056

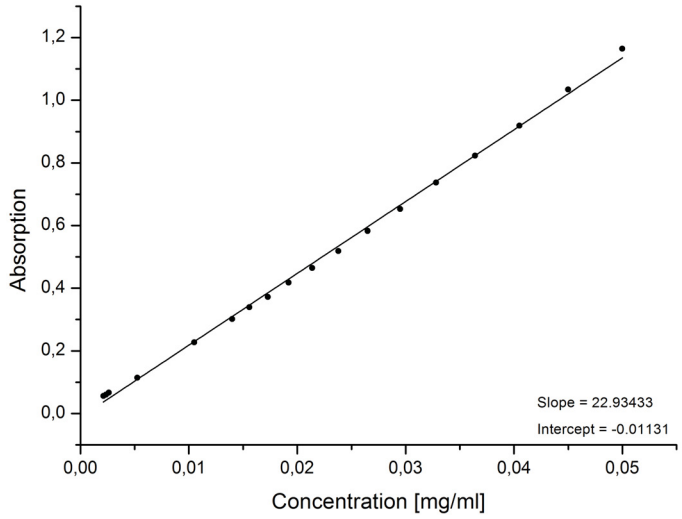


Figure A.6.1. Calibration curve of carvedilol with a linear regression.

Figure A.6.1 represents the obtained calibration curve with a linear fitting.

Table A.6.2. Statistics of the linear regression of the calibration curve of Car.

Multiple correlation coefficient	0.9991
Coefficient of determination	0.9983
Adjusted coefficient of determination	0.9982

Despite good correlation statistic parameters (table A.6.2), the residuals plot of this linear regression (figure A.6) indicates a systematic error and suggests a modelling by a polynomial of higher order.

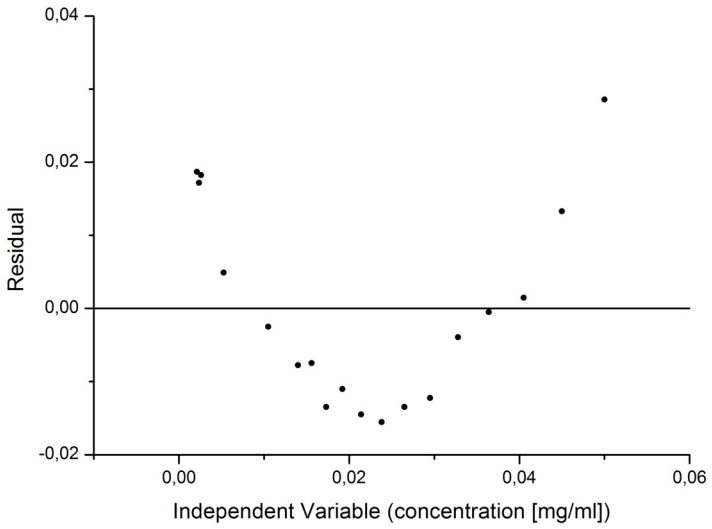


Figure A.6.2. Residuals plot of the linear regression for the calibration curve of Car.

A quadratic polynomial fitting is hence used for the calibration curve (figure A.6). The obtained adjusted coefficient of determination is satisfactory as well as the residuals plot (figure A.6).

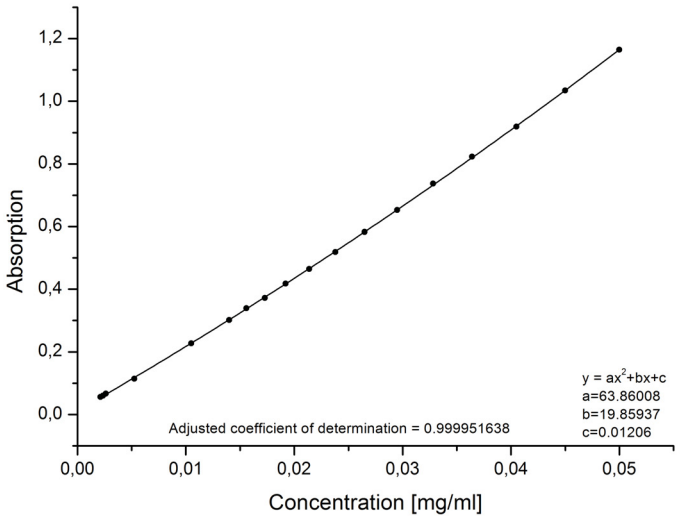


Figure A.6.3. Calibration curve of carvedilol with a quadratic polynomial regression.

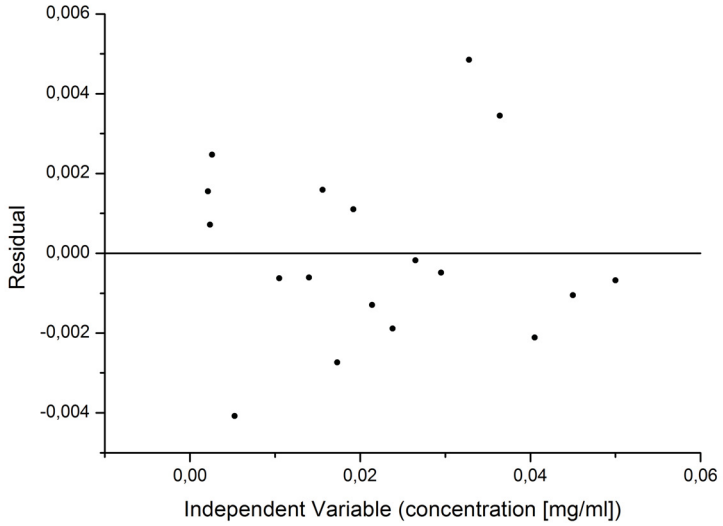


Figure A.6.4. Residuals plot of the quadratic polynomial regression for the calibration curve of carvedilol.

A.7. Calculation of the absolute humidity

For the purpose of stability tests, the relative humidity and the temperature were recorded. In order to compare humidity values independently from the temperature the absolute humidity was calculated as follows [88]:

- Equation A.1 describes the calculation of the absolute humidity:

$$A = C\,\frac{P_w}{T} \tag{A.1}$$

  where A: absolute humidity in g/m^3; $C = 2.166\,79\,\mathrm{g\,K/J}$ (constant), ideal gas behaviour is assumed; P_w: vapour pressure in Pa; T: temperature in K.

- P_w can be calculated using equation A.2:

$$P_w = P_{ws}\,\frac{RH}{100} \tag{A.2}$$

  where P_w: vapour pressure in hPa; P_{ws}: saturation vapour pressure in hPa; RH: relative humidity in %.

- P_{ws} can be calculated with the simplified equation A.3:

$$P_{ws} = a \times 10^{\frac{bT}{T+c}} \tag{A.3}$$

  where P_{ws}: saturation vapour pressure in hPa; $a = 6.116\,441$ hPa (constant); $b = 7.591\,386$ (constant); T: temperature in °C; $c = 240.7263$ K (constant).

Here is an example of calculation: Absolute humidity of milling conditions (table A.7.1):

Table A.7.1. Surrounding conditions during ball milling of samples

Temperature [°C]	**Relative humidity [%]**	**Absolute humidity [g/m^3]**
5.0 ± 0.5	90.0 ± 10.0	6.1

$$P_{ws} = a \times 10^{\frac{bT}{T+c}} = 6.116\,441\,\mathrm{hPa} \times 10^{\frac{7.591386 \cdot 5}{5+240.7263}} = 8.729\,\mathrm{hPa}$$

$$P_w = P_{ws}\frac{RH}{100} = 8.73\,\mathrm{hPa} \cdot \frac{90}{100} = 7.856\,\mathrm{hPa}$$

$$A = C \cdot \frac{P_w}{T} = 2.166\,79\,\mathrm{hPa} \cdot \frac{785.6\,\mathrm{Pa}}{278.15\,\mathrm{K}} = 6.1\,\mathrm{g/m^3}$$

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Eiman Atiek
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