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„ The influence of glass transition temperature and molecular interactions on the stability of co-amorphous systems “

verfasst von / submitted by

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Abstract

It has been estimated that around 90% of low molecular weight pharmaceutical drugs in development show poor aqueous solubility, which often leads to low or variable oral absorption, resulting in a low bioavailability. Therefore, new strategies to overcome the issue of low dissolution rate and lack of solubility are being investigated in academia and by the pharmaceutical industry. Co-amorphization, converting two crystalline compounds into one co-amorphous mixture, has been shown to be a feasible approach to create new pharmaceutical samples that improve dissolution rate and solubility of poorly water-soluble drugs, but also the physical stability of amorphous drugs during storage.

This thesis focuses mainly on the question, whether the stability of co-amorphous compounds at equimolar ratios, stored at different temperatures is mainly dependent on their glass transition temperatures and/or on molecular interactions. In this study, one non-polar amino acid (phenylalanine) was chosen as a co-former to interact with eleven different drugs (carvedilol, cimetidine, celecoxib, furosemide, ibuprofen, indomethacin, mebendazole, naproxen, nitrofurantoin, paracetamol, simvastatin) by different molecular interactions forming co-amorphous binary mixtures.

In the first part of this study, the role of phenylalanine (PHE) as a suitable co-former was investigated. Co-amorphous forms of phenylalanine (PHE) and the drugs were prepared by vibrational ball-milling at four different molar ratios each (25% drug – 75% PHE, 50% drug – 50% PHE, 75% drug – 25% PHE, pure substance). X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) revealed that almost all mixtures possessed a halo in their diffractograms and a single glass transition temperature (Tg) in the DSC measurements, indicating that they were successfully converted into their co-amorphous forms. The Fourier transform infrared spectroscopy (FTIR) results indicated molecular interactions, that were responsible for forming and maintaining the co-amorphous state.

The second part of this study was the stability assessment of co-amorphous samples at equimolar ratios. The samples were stored at different storage temperatures, also defined as “controlled conditions” in this thesis (room temperature (RT), 40°C, 80°C) and their solid states were measured using XRPD to monitor the onset of recrystallization at regular intervals. PHE was one of the

few samples that could not be converted into its pure amorphous form and so a Tg could not be measured with the DSC. Therefore, the Fox equation was used (when contrasted with the experimentally determined Tgs) and rearranged with respect to the Tg of PHE to calculate a hypothetical Tg of PHE for every equimolar co-amorphous form. The values of the calculated hypothetical Tgs of PHE were used to indicate how strong the molecular interactions would be between each drug and PHE. Those molecular interaction from the FTIR measurements were then later correlated to the stability results of the co-amorphous samples.

It was found that almost all binary formulations stayed physically stable at RT throughout the entire study. Two drug-PHE mixtures with "low" and "medium" Tgs revealed the onset of recrystallisation for one of the compounds in the blends, while the other one did not recrystallize. Samples with higher Tgs and calculated stronger interactions between the drugs and the amino acid (AA) showed lower stability than expected at storage temperatures (80°C) close to their Tgs and recrystallised within days. There was only one co-amorphous form that stayed physically stable for the entire period of the study at both conditions, RT and 40°C. Simultaneously the other co-amorphous samples of drug-PHE at molar ratios of 25% drug – 75% PHE, of 75% drug – 25% PHE and pure amorphous drugs were stored at RT, also defined as "uncontrolled conditions" in this thesis. The idea of this attempt was to see if co-amorphous mixtures of drug-AA at different molar ratios would be more useful than co-amorphous mixtures at equimolar ratios, in terms of the time of stability.

In conclusion, the study described in this thesis shows that the time of stability of co-amorphous samples at equimolar ratios can not be attributed to their measured Tgs and / or strength of molecular interactions within the co-amorphous systems only. New findings suggest, that the choice of an equimolar blend might not always be the best one to produce co-amorphous forms, that retain their physical and chemical stability for a longer period.

List of abbreviation

AA	Amino acid
API	Active pharmaceutical ingredient
BCS	Biopharmaceutic classification system
BM	Ball mill
CAR	Carvedilol
CIM	Cimetidine
CEL	Celecoxib
Cp	Heat capacity
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
FUR	Furosemide
GT	Gordon-Taylor equation
IBU	Ibuprofen
IND	Indomethacin
MEB	Mebendazole
MW	Molecular weight
NAP	Naproxen
NIT	Nitrofurantoin
PAR	Paracetamol
PHE	Phenylalanine
RT	Room temperature
Tg	Glass transition temperature
SIM	Simvastatin
XRPD	X-ray powder diffraction

1. Introduction

Oral administration of drugs has been the favoured route of administration for reasons of higher convenience and thus patient compliance, no need for sterile conditions and cost-effective production (Sastry *et al.,* 2000). In the past years it has been verified by many studies, that around 40% of drugs on the market as well as a large number of drug substances (up to 90%) in development appear to be poorly water-soluble and possess a low dissolution rate (Blaabjerg *et al.,* 2017; Huang *et al.,* 2016). This unfortunate situation leads to a vicious circle of low drug absorption after oral application and simultaneously a low bioavailability (Van Drooge *et al.,* 2006; Löbmann *et al.,* 2013). One way of evading this challenge in drug development would be to increase the dose and dosing frequency of the active pharmaceutical ingredient (API) until the therapeutic drug concentration is reached, which in consequence would result primarily in manufacturing difficulties and high manufacturing costs, but more importantly in unwanted and toxic side effects, ensuing a reduced compliance amongst patients (Kawabata *et al.,* 2011). Therefore, the pharmaceutical industry as well as many academic research groups are focused on finding ways to overcome the issues of slow dissolution rate and poor aqueous solubility of poorly water-soluble drugs.

To achieve high bioavailability, a drug has to be absorbed and should have the following two characteristics: high dissolution rate and solubility in the fluids of the gastrointestinal (GI) tract and sufficient permeation through the GI tract epithelial membranes (Mu *et al.,* 2013). Amidon and co-workers developed a classification scheme that organises drugs in four groups with regards to their dissolution and permeation properties, and named it the biopharmaceutics classification system (BCS) (Amidon *et al.,* 1995) (Table 1). Drug substances are classified as follows:

Table 1. The biopharmaceutics classification system

	High Solubility	**Low Solubility**
High Permeability	Class I	Class II
Low Permeability	Class III	Class IV

The U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) has set some guidelines on how to determine solubility and permeability of drugs which can be found on the following website: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/waiver-vivo-bioavailability-and-bioequivalence-studies-immediate-release-solid-oral-dosage-forms. Plöger *et al.*, have used those guidelines to determine the solubility of several active pharmaceutical ingredients (API) (Plöger *et al.*, 2018):

→ A "highly soluble" drug is classified as a substance of which the highest clinical dose is soluble in ≤ 250 mL of aqueous media within the pH range of 1 - 6.8 at 37 ± 1°C.

→ A drug is classified as "highly permeable" when the extent of absorption or systemic bioavailability in humans is ≥ 85% of the administered dose. The presence of stability of the drug in the gastrointestinal tract must be evident.

Comparing the groups, it is evident, that class I drugs possess high permeability as well as high solubility and are therefore the most convenient ones for oral drug delivery. Class II drugs contain high permeability, but poor oral bioavailability might be linked to limited dissolution and low solubility. Class III drugs have high solubility but their absorption (and thus oral bioavailability) is often inadequate due to their low permeability. Class IV drugs have both low permeability and low solubility and are therefore usually not suitable or recommended for the use as oral dosage forms.

Benet and co-workers estimated that 70% of new drugs are identified as BCS class II drugs (Benet *et al.*, 2011) and this has necessitated the development of new delivery options for poorly water-soluble drugs such as crystal modification, particle size reduction, pH modification and amorphization to improve their solubility and dissolution rate (Kawabata *et al.*, 2011).

Amorphization, i.e. converting a crystalline compound into its amorphous analogue, an approach used in this study, has gained interest in pharmaceutical drug development due to an enhanced solubility and as a result higher bioavailability of this particular solid state of matter (Jensen *et al.*, 2014). Amorphous and crystalline forms differ in their thermodynamic properties when heating or cooling the materials, as shown in Figure 1. When heated, the

crystalline drug is starting to melt at its defined melting point (Tm), which is a thermodynamic property. For the following step, two scenarios can occur. Scenario A: The molten substance is cooled down at a slow rate and its molecules have enough time to rearrange and recrystallize into a thermodynamically stable (or metastable) crystalline form (Kawakami and Pikal 2005). Scenario B: The melt is cooled sufficiently quickly to temperatures below its melting point, and thus the substance reaches the super cooled liquid state (without crystallization) but being still in equilibrium with the melt (Graeser *et al.*, 2010). Upon further temperature decrease the super-cooled liquid state falls out of equilibrium (with regard to the molten drug) until it reaches the glass transition temperature (Tg) and thus attains an amorphous form, the so-called glassy state. X-ray powder diffraction (XRPD) is used to verify the process of amorphization by the presence of a characteristic "halo" and lack of diffraction peaks. The Tg of an amorphous substance can easily be determined with differential scanning calorimetry (DSC) by evaluating a step change in the heat capacity (ΔCp).

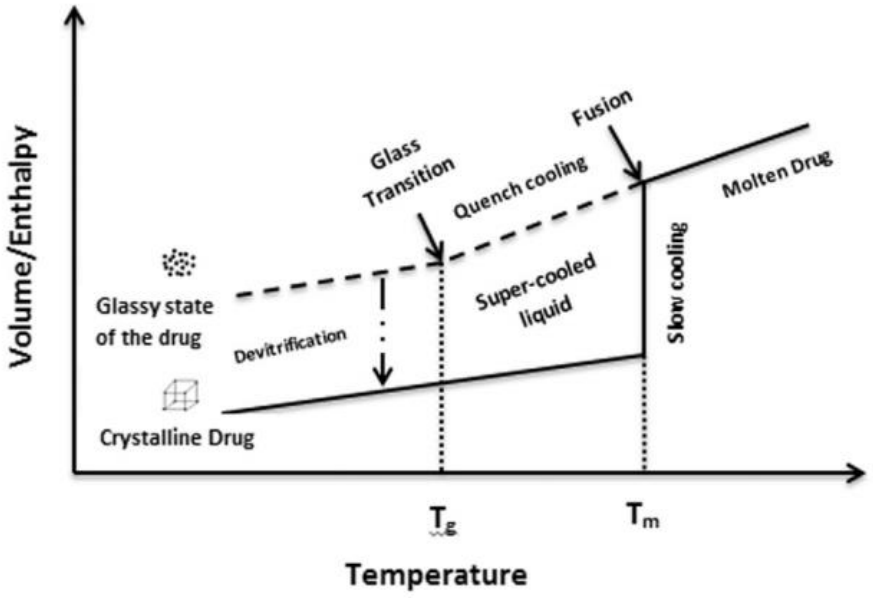


Figure 1. Change in enthalpy and volume as a function of temperature. Reprinted from Baghel *et al.*, with permission (Baghel, *et al.*, 2016).

1.1. Amorphous drugs and amorphous solid dispersions

As stated above, one promising approach to enhance the effectiveness of new drugs is to convert them from a crystalline state to the corresponding amorphous form (Löbmann *et al.*, 2013). There are two possible ways to attain the amorphous state: the thermodynamic pathway and the kinetic pathway (Blaabjerg *et al.*, 2017).

The thermodynamic pathway describes the transformation of a material from a crystalline solid into either its molten state or a solution (usually in an organic liquid), also described as "thermodynamically stable disordered states". The melt is then quench cooled quickly, or the organic solution evaporated rapidly, in order to reach the amorphous state (Blaabjerg *et al.*, 2017).

The kinetic pathway, also called mechanical activation, implies a rising amount of crystal defects, for example by vibrational ball-milling, until the amorphous state is reached (Hancock and Zografi 1997). The following results apply for both pathways: the long-range order that is found in crystalline material does not exist in the amorphous state. The irregular arrangement of molecules in amorphous solids is similar to that of liquids and amorphous forms are therefore often described as a "solid with liquid-like properties" (Hancock and Zografi 1997). It is also important to mention that the choice of a certain preparation technique needs to be suitable for the drug substance. For instance, heat-based methods are not a good way to convert thermolabile drugs into their amorphous form. In contrast, ball-milling might not transfer enough energy to some crystalline materials for full amorphization.

Whilst the advantages of developing amorphous pharmaceuticals are, as mentioned above, their potentially higher bioavailability after oral administration, because of increased mobility and energy within that state, and thus a higher solubility and a higher dissolution rate (Strachan *et al.*, 2015), their downside lies in their physical instability. Individual amorphous drugs usually lack physical stability during preparation, storage or even administration and tend to transform back to their more stable corresponding crystalline form(s), i.e. amorphous forms are thermodynamically unstable (Löbmann *et al.*, 2013). Therefore, different approaches were made to find methods preventing (or at least slowing down) the process of recrystallisation and produce practically stable amorphous systems of

drugs to benefit from their advantages. Those systems are called amorphous solid dispersions (ASD).

By combining the drug with a second compound, stabilization of the amorphous form can be attained. Solid dispersions, first defined by Chiou and Riegelman, are characterized as mixtures of poorly aqueous soluble drugs and inert carriers, existing on molecular or particulate level (Chiou and Riegelman, 1971; Vasconcelos *et al.*, 2007). Figure 2 shows a complex classification system of solid dispersions. One form of solid dispersions are ASDs, also called glass solutions. These are single phase amorphous systems with at least two components and can be organized by the nature of the stabilising excipients, for example polymeric or non-polymeric excipients (mesoporous silica-based glass solutions and co-amorphous mixtures).

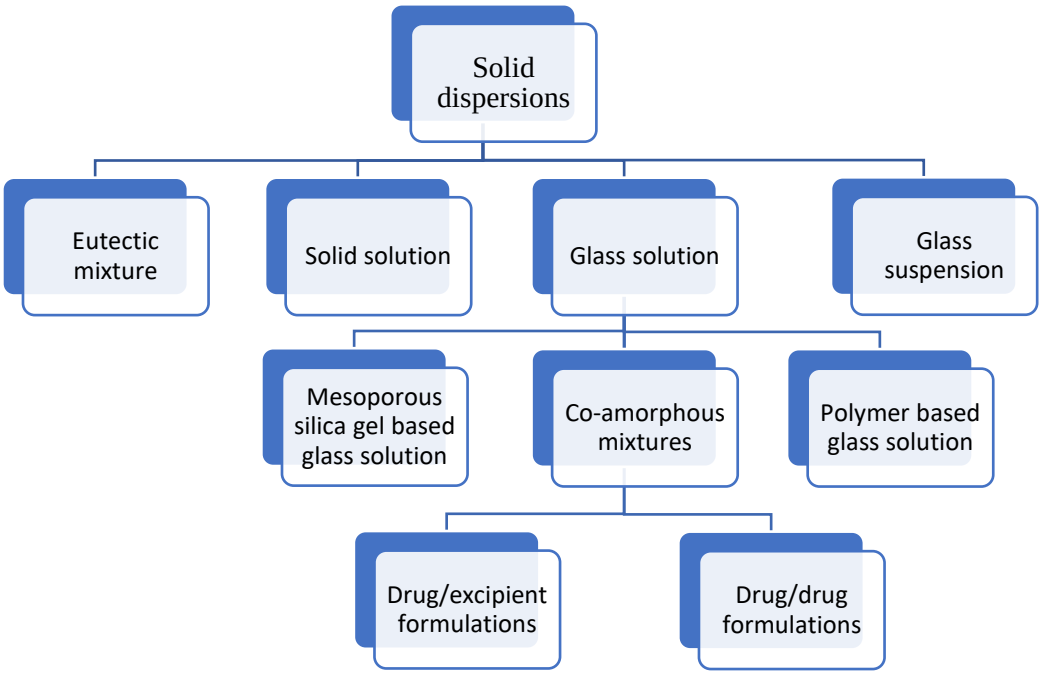


Figure 2. Classification scheme of solid dispersions – Solid dispersions represent a group of four different categories of solid mixtures with unique characteristics with respect to their number of components, phases and solid-state nature. Eutectic mixtures consist of two components with two phases that are both crystalline. Similar to glass solutions are solid solutions, prepared with one or more solutes in a solvent but, in contrast to glass solutions, one crystalline phase. Glass suspensions comprise of amorphous or crystalline drugs in amorphous carriers (modified from Dengale *et al.* (Dengale *et al.*, 2016).

Polymer-based glass solutions consist of polymeric carriers that stabilize the amorphous form of the drug while the drug is dissolved molecularly in the polymer (Van Den Mooter 2012). The improved physical stability of those systems can have the following reasons: Polymers possess a high Tg and therefore the Tg of the polymer-based glass solutions is expected to be higher compared to the Tg of the amorphous drug alone, and thus the molecular mobility of the drug molecules decreases (Vasconcelos *et al.,* 2007). An additional bonus to prevent recrystallisation of the drug are molecular interactions between the functional groups of both components, which usually are either hydrogen bond donors or acceptors (Janssens and Van den Mooter 2009). Unfortunately, one mayor drawback of polymeric ASDs is the hygroscopic nature of most polymers. Mostly used polymers include PVP, PVP/VA, HPMC, HPMCAS, different Eudragits and Soluplus (Shamma and Basha 2013; Sun *et al.,* 2010). The absorbed water, especially during storage, from water in the gas phase, acts as a plasticizer, which has a negative effect on the stability of the ASD, reducing the Tg and thus increasing molecular mobility, resulting in phase separation and recrystallisation (Rumondor and Taylor 2010). That is why still today considerable research is needed to find new ways of stabilizing amorphous drugs.

1.2. Co-amorphous systems

Recently there has been a growing interest in manufacturing co-amorphous mixtures consisting of molecules with low molecular weight, as an alternative to polymer-based ASDs. Chieng *et al*. developed a binary system that consisted of two drugs, ranitidine hydrochloride and indomethacin, to evaluate their physicochemical properties and stabilities (Chieng *et al*., 2009). The concept proved to be successful, as both drugs were able to stabilize each other, and therefore the term “co-amorphous” was introduced, representing an amorphous system consisting of only low molecular weight, initially crystalline, compounds. This contrasts with polymer-based amorphous mixtures, where the polymer is usually already amorphous (Strachan *et al*., 2015).

When producing a co-amorphous system, two types of co-formers can be used: a) drugs (Löbmann *et al.,* 2012) and b) low molecular weight excipients. Drug-drug combinations consist of two pharmacologically relevant drugs, that can be administered together in therapy and both act as active pharmaceutical

ingredients and excipients at the same time. Some examples of successful studies on drug-drug co-amorphous compounds with greater physical stability and dissolution rate compared to the pure amorphous drugs are indomethacin and naproxen (Löbmann *et al.*, 2011), simvastatin and glipizide (Löbmann *et al.*, 2012) and carvedilol and atorvastatin (Shayanfar and Jouyban 2013).

The other form of producing a co-amorphous system is by stabilizing the drug with excipients, such as amino acids (AA) (Löbmann *et al.*, 2013; Jensen *et al.*, 2014), organic acids (Fung *et al.*, 2018; Wu *et al.*, 2018), and sugars *(Chavan et al.*, 2016). Several studies have been published showing that AA have great potential to act as co-formers stabilizing the co-amorphous form (Jensen *et al.*, 2014, 2016; Kissi *et al.*, 2018). A large range of AAs with unique physicochemical properties is available offering several options for specific co-formers to choose from.

Co-amorphous systems are usually prepared at an equimolar molar ratio involving the crystalline drug in question and its crystalline co-former *(Kasten et al.*, 2016). Figure 3 contrasts the two most important factors of BCS class II crystalline drugs compared to the equivalent amorphous form: stability and solubility. By adding a suitable co-former to the crystalline drug and a subsequent suitable amorphization, one can achieve a stabilizing effect by different molecular interactions between the two components, such as hydrogen bonding (Löbmann *et al.*, 2013), π-π interactions (Löbmann *et al.*, 2013) and salt formations (Yamamura *et al.*, 2002), as well as an enhanced dissolution rate and solubility (Gupta *et al.*, 2004).

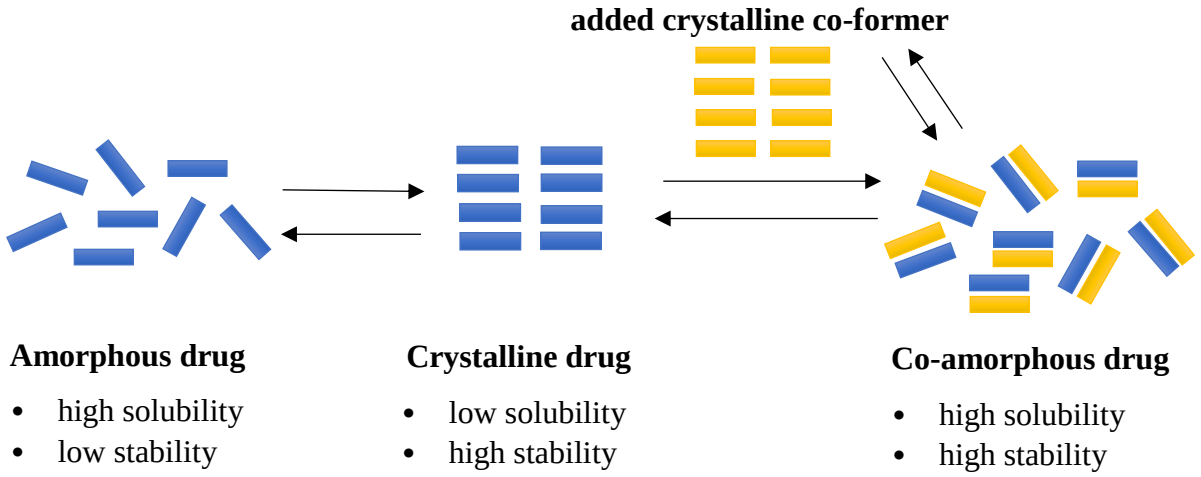


Figure 3. Differentiation between amorphous drugs, crystalline drug and co-amorphous drug. Drug molecules are indicated in blue, co-former molecules are indicated in yellow.

The following three important factors must be taken into consideration when choosing a suitable co-former to develop the desired co-amorphous system: 1) co-formability, 2) physical stability and 3) dissolution and solubility enhancement.

1) Co-formability

The term co-formability describes the ability of the tested co-former to easily form a co-amorphous mixture with the drug in question (Wu *et al.,* 2018). Kasten *et. al* performed a study on developing a screening method for co-amorphous substances, containing six drugs and 20 AAs (Kasten *et al.,* 2016). The results showed that non-polar AAs (e.g. tryptophan, phenylalanine, valine, proline) would be a good first choice for a co-former to attain a co-amorphous form with a drug. Basic AAs (e.g. arginine, histidine, lysine) turned out to be appropriate for amorphous salt formations when combined with acidic drugs. Acidic AAs (e.g. aspartic acid, glutamic acid, serine, glycine) though seem to be unsuitable co-formers as they showed poor co-formability, which was ascribed to their polar properties (Meng-Lund *et al.,* 2018). In this thesis ball-milling was used to prepare the (co)-amorphous samples and co-formability was assessed based on two factors: whether the system was able to achieve complete miscibility with no signs of remaining crystallinity of each substance, hence forming a single-phase co-amorphous system (Dengale *et al.,* 2016) and the ball-milling time needed to accomplish complete amorphization.

2) Physical stability

It is a known fact, that amorphous drugs have a propensity towards recrystallisation and that their long-term physical stability upon storage has become one of the focuses in academic and industrial pharmaceutical research. The use of suitable co-formers has become largely inevitable as studies showed positive as well as negative results regarding the physical stability of co-amorphous blends (Lim *et al.,* 2016; Newman *et al.,* 2017).

3) Dissolution and solubility enhancement

As previously mentioned, BCS class II and class IV drugs are affected by poor aqueous solubility and / or a low dissolution rate, both important quality parameters in the development of pharmaceutical solid forms. Studies have shown that pharmaceuticals in the (co-)amorphous state display a notably better dissolution rate and water solubility (oftentimes further enhanced by adding a co-former) than their crystalline equivalents (Wu *et al.*, 2018) .

1.3. Analytical experimental techniques used in this thesis

To carry out this study, all samples were prepared by ball-milling and a range of analytical techniques was used to define the solid state of the samples as well as their characteristics, such as amorphousness, molecular interactions and stability.

Table 2. A list of methods, as well as their aim, that were used in this study.

Methods	Aim
Ball-milling	What is the needed ball-milling time to reach the amorphous state?
XRPD	Was the process of amorphization successful?
DSC	Does the thermogram show a step change in the sample´s heat capacity, indicating the presence and position of a glass transition temperature, Tg?
FTIR	Do the spectra confirm molecular interactions e.g. hydrogen bonding or π-π interactions?

1.4. Thesis aim

The aim of this study was to systematically investigate, whether the Tg and / or the presence of molecular interactions influenced the stability of co-amorphous systems of a drug and AA at an equimolar ratio.

The boundary conditions of this study were as follows:

- As an excipient and common denominator, PHE was chosen as the AA, as it is a neutral, aromatic, non-polar AA featuring a hydrogen donor group, and is therefore a good co-former (Kasten *et al.*, 2016)
- Eleven drugs were chosen: furosemide, celecoxib and indomethacin as acidic drugs; mebendazole, carvedilol and cimetidine as basic drugs; nitrofurantoin and simvastatin as neutral drugs. The drugs were selected by their hydrogen acceptor ability, pH values and difference in their individual Tgs and were divided into "high-Tg", "medium-Tg" and "low-Tg" groups.
- For all binary systems it was important, that there is the possibility of some sort of interactions (hydrogen bonding, π-π stacking), but no salt formation should occur as that would constitute a new compound, held together by ionic bonds.

This study was focusing on the stability of co-amorphous samples stored at specific storage temperatures to monitor the onset of recrystallization. Our hypotheses are:

a) Interactions, like hydrogen bonding or ionic bonding between drugs and excipients are responsible to stabilize the co-amorphous samples;

b) The values of Tgs are important reference values when predicting the stability of co-amorphous samples.

The goal was first to see, whether the functional groups of PHE (e.g. carboxylic group, amino group) could interact with the drugs in a co-amorphous mixture and therefore could be used as a stabiliser for the amorphous drugs, inhibiting their crystallization, and secondly, whether the stability of those binary mixtures is mainly dependent on those molecular interactions or the Tg of the resulting co-amorphous form.

2. Materials and Methods

2.1. Drugs and amino acid used for this study

Table 3. A list of all the drugs and the AA, including their molecular weight, supplier and indication, used in this study. The chemical structures are shown on the next page.

Drug name, abbreviation used in this study	**Molecular weight (M_W) ($g*mol^{-1}$)**	**Supplier**	**Indication**
Carvedilol, CAR	406,48 g/mol	Cipla Ltd. (Mumbai, India)	ß-blocker
Celecoxib, CEL	381,37 g/mol	Fagron Inc. (St. Paul, MN, USA)	selective COX_2 inhibitor
Cimetidine, CIM	252,34 g/mol	Hawkins Inc. (Minneapolis, MN, USA)	H_2 Receptor inhibitor
Furosemide, FUR	330,74 g/mol	Sigma-Aldrich (St. Louis, MO, USA)	Loop diuretic
Ibuprofen, IBU	206,29 g/mol	Sigma-Aldrich (St. Louis, MO, USA)	NSAID
Indomethacin, IND	357,79 g/mol	Fagron (Copenhagen, DNK)	NSAID
Mebendazole, MEB	295,29 g/mol	Sigma-Aldrich (St. Louis, MO, USA)	Anthelminthic microtubule inhibitor
Naproxen, NAP	230,26 g/mol	Sigma-Aldrich (St. Louis, MO, USA)	NSAID
Nitrofurantoin, NIT	238,16 g/mol	Unikem (Copenhagen, DNK)	Therapy of urinary tract infection
Paracetamol, PAR	151,16 g/mol	Fagron Services BV. (Uitgees, NL)	Pain reliever and fever reducer
Simvastatin, SIM	418,57 g/mol	Hangzhou Dayangchem CO. Ltd. (Hangzhou, China)	HMG-Co A reductase inhibitor
L-Phenylalanine, PHE	165,19 g/mol	Sigma-Aldrich (St. Louis, MO, USA)	Amino acid

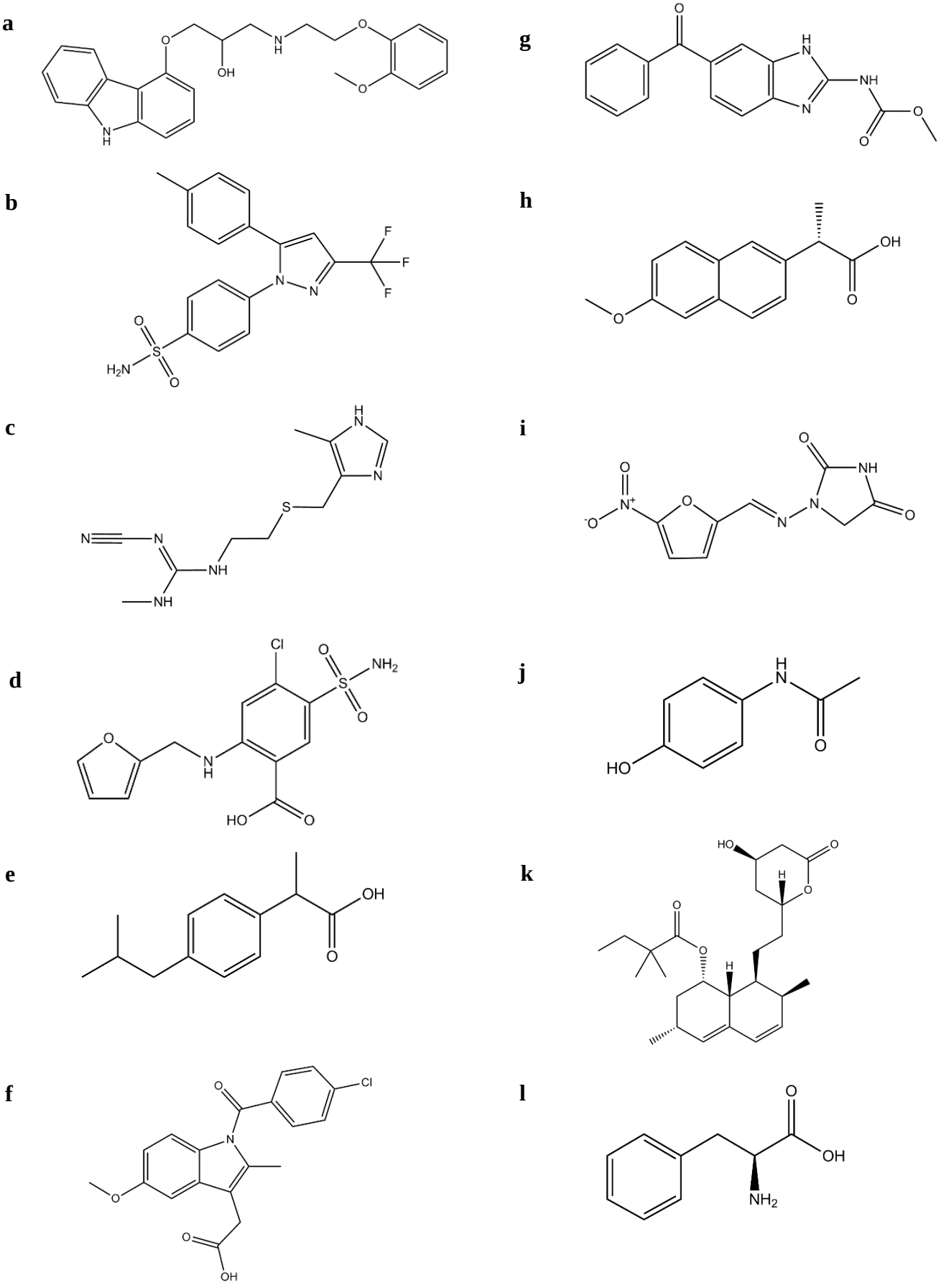


Figure 4. Chemical structures of carvedilol (a), celecoxib (b), cimetidine (c), furosemide (d), ibuprofen (e), indomethacin (f), mebendazole (g), naproxen (h), nitrofurantoin (i), paracetamol (j), simvastatin (k), phenylalanine (l).

2.2. Preparation and characterisation of co-amorphous samples

2.2.1. Preparation of amorphous materials

The formation of (co-)amorphous mixtures was accomplished by mechanical activation (ball milling) that deforms the crystal lattice and by mixing the components, converting their state from crystalline to (co-)amorphous (Karagianni *et al.,* 2018). A total mass of 1000 mg powder of the crystalline starting materials (drug-AA) was turned into co-amorphous systems by ball-milling for 90 minutes or longer if needed. The samples were milled in an oscillatory ball mill (Mixer mill MM400, Retch GmbH & Co., Hann, Germany) at a frequency of 30 Hz in 25 ml jars containing two stainless steel balls with a diameter of 12 mm. The mill was placed in a cold environment at 6°C. Four different mixtures were prepared for each of the 11 co-amorphous combinations by varying the molar ratio between the drug and the AA (containing 25, 50, 75, 100 mol% drug) and were then further analysed on the day of preparation.

2.2.2. X-ray powder diffraction (XRPD)

To determine whether the mixtures turned fully amorphous or not, XRPD measurements were performed using an X´Pert PANalytical PRO X-ray diffractometer (PANalytical, Almelo, The Netherlands) with CuKα radiation (1.54187 Å), acceleration voltage of 45 kV and a current of 40 mA. The samples were scanned in reflectance mode between 5° and 35° 2θ with a scan rate of 0.067335° 2θ/s and a step size of 0.0262606°. The data were collected and analysed using the software X´Pert HighScore Plus (PANalytical, Almelo, The Netherlands).

2.2.3. Differential scanning calorimetry (DSC)

DSC measurements were performed on all ball-milled samples with a Discovery DSC (TA-Instruments-Waters LLC, New Castle, USA). The samples, weighing 3-6 mg, were analysed in closed Tzero aluminium pans. The thermal behaviour of the samples was measured in a modulated temperature mode, with measurements ranging from -10°C to 200°C, a heating rate of 2 °C/min, an amplitude of 0.21°C and a period of 40 s. Independent triplicate measurements were performed and the experimental Tgs were recorded as the midpoints of the step change in the

reversing heat flow signals using Trios software (TA Instruments, New Castle, USA) and then calculated as the mean of each independent triplicates.

2.2.4. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopic measurements of the mixtures were carried out to investigate the molecular interactions between drugs and AA at a 1:1 molar ratio. A MB3000 FTIR (ABB, Quebec, Canada) equipped with a MIRacle® ATR sampling accessory with a ZnSe crystal (PIKE Technologies, Madison, USA) was used to collect the spectra from 400-4000 cm^{-1} as a mean of 64 spectra with a resolution of 4 cm^{-1}. The infrared spectra were collected with Horizon MB® software (version 3.2.5.2, ABB, Quebec, Canada) and analysed in the region of 800-1800 cm^{-1} as well as 2300-3500 cm^{-1} using the OriginPro 2019 software.

2.2.5. Calculated hypothetical Tgs values using the Fox equation

PHE alone could not be made amorphous and so it was not possible to determine its experimental Tg by DSC measurements. This also excluded the use of the Gordon-Taylor equation to calculate the theoretical Tg of PHE, as we would need the measured Tg but also the density of the amorphous compound. Therefore, a *hypothetical* Tg of PHE was calculated for every binary co-amorphous mixtures using the Fox equation after rearranging it with respect to the Tg of the AA. This is a novel approach to look for interactions not based on deviation to the Gordon-Taylor equation or Fox equation, but in the case where one Tg is not available, to determine a "hypothetical Tg" of that compound. The calculations included the weight fractions of PHE and the drug, respectively, as well as the experimentally determined Tgs of the co-amorphous samples and amorphous drugs.

2.2.6. Physical stability upon storage

The binary co-amorphous systems at equimolar ratios were stored in desiccators under dry conditions (silica gel) at RT (20-25°C), 40 °C and 80°C, also defined as "controlled conditions" in this thesis. It is a known fact, that humidity affects the thermal properties of amorphous compounds, acting as a plasticizer and lowering the Tg (Rumondor and Taylor 2010). For this study however, only dry conditions were considered, to be able to focus on the influence of the Tgs of the co-amorphous samples on stability. To monitor the onset of recrystallization of

drugs and the AA, samples were analysed using XRPD every three days for the first month, twice a week for the second month and then weekly for the last month. Another set of binary co-amorphous samples at different molar ratios (25% drug – 75% AA, 25% AA – 75% drug) as well as the pure amorphous drugs were stored in a storage box at RT and are defined as samples stored under "uncontrolled conditions", to compare both sets of samples in regard to stability outcomes due to different molar ratio choices.

3. Results and discussion

3.1. Determination of successful amorphization by XRPD

XRPD was used to verify whether the pure drugs and drug-AA mixtures at different ratios turned (co-)amorphous after ball-milling. Besides the time for ball-milling the samples, it is also important to keep track of the temperature of the surrounding area during the ball-milling process: to prevent quick recrystallization during the milling process, it is recommended to carry out the milling at low temperatures (4-6°C) in order to reach the amorphous state easier (Shi *et al*., 2019). Figure 5 shows an example of the diffractograms of the amorphous and crystalline forms of the pure drug indomethacin. An XRPD diffractogram with no peaks and a characteristic halo pattern is a strong indicator of a successfully achieved amorphization (Kissi *et al.*, 2018). The results for all investigated compounds and mixtures are shown in section 6.3. “XRPD DATA”, comparing the diffractograms of the crystalline drugs, the crystalline AA and the respective co-amorphous forms of drug-AA mixtures before evaluating their stability and the diffractograms showing the onset of recrystallization during the stability study.

Out of all 35 samples that were ball-milled for 90 minutes or longer, only five could not be transformed into the (co-)amorphous state. The diffractograms of SIM-PHE at the 25:75 molar ratio, of IBU-PHE, NAP-PHE and PAR-PHE at equimolar ratio, and of pure PHE, showed even after several hours of ball-milling some remaining X-ray diffractions that could be related to the starting crystalline form of the drug as well as the crystalline AA.

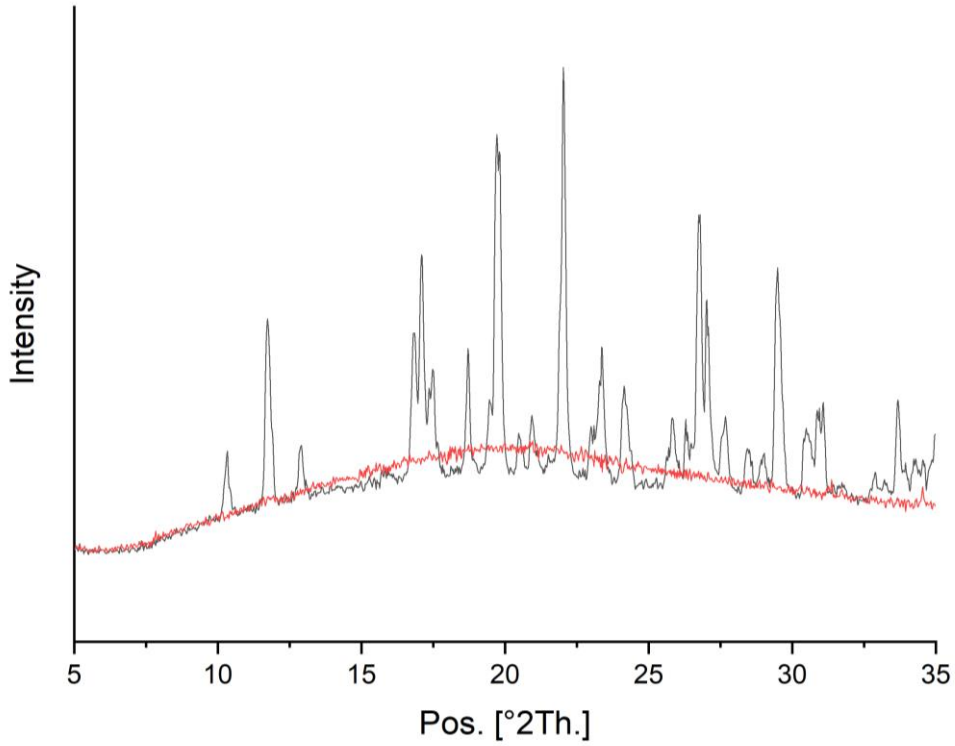


Figure 5. XRPD diffractograms of crystalline IND (shown in black) in comparison to its amorphous form (shown in red) after 90 minutes of ball milling.

It can be stated that these mixtures do not become (co)-amorphous or recrystallize quickly during the ball-milling process. It is possible that the preparation technique was not the best choice for amorphization of these mixtures, and that different results may have been achieved e.g. using spray drying (Mishra *et al.*, 2018). The further attempt to reach amorphization for pure PHE through quench cooling also failed because the sample began to degrade during the melting process. Another study reported the same problem for PHE and two other amino acids (tryptophan and arginine) (Löbmann *et al.*, 2013).

However, the majority of samples investigated confirmed previous studies, where the use of non-polar AA, such as tryptophan, phenylalanine, proline and valine, was a good choice when forming co-amorphous mixtures with a drug (Löbmann *et al.*, 2013; Kasten *et al.*, 2016). The diffractograms of those binary mixtures showed no peaks, indicating that (co-)amorphization was successful after the appropriate time of ball-milling.

3.2. Determination of thermal properties by DSC

Differential scanning calorimetry is a thermal analytical technique that detects how a material's heat capacity (Cp) changes as a function of temperature and time (Gill *et al.*, 2010). It controls both, heating and / or cooling of the sample in question parallel to a reference sample (or an empty pan) inside a DSC furnace and detects changes in the heat capacity as changes in the heat flow (Paudel *et al*, 2014). Transitions like melting points (Tm), glass transitions (Tgs), phase changes, and curing process can be measured and evaluated in the resulting thermograms.

The Tgs of the co-amorphous mixtures as well as of the pure amorphous drugs were analysed by DSC and the results revealed that all co-amorphous mixtures, at different molar ratios of drug-PHE, showed a single Tg in the DSC thermogram, indicating that they were molecularly homogeneous (Huang *et al.*, 2016) (Figure 6). As pure crystalline PHE could not be turned into an amorphous form by ball-milling, it was not possible to determine the Tg of the pure AA.

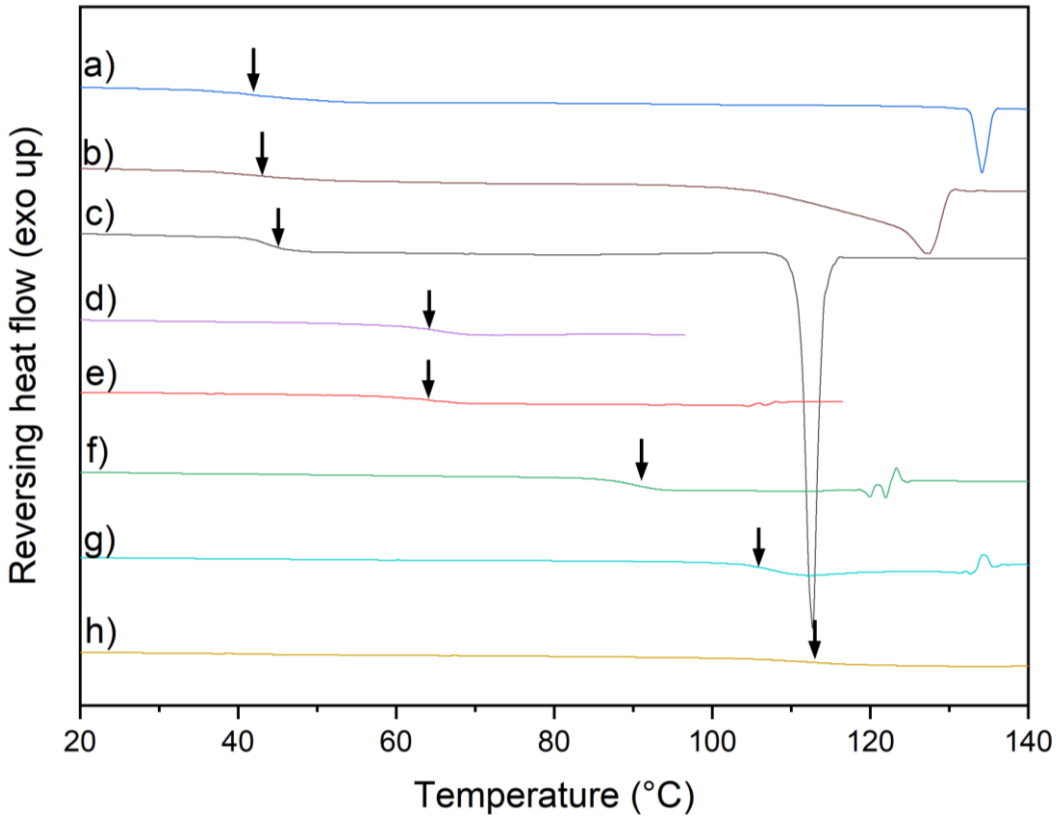


Figure 6. DSC thermograms of co-amorphous a) CIM-PHE, b) SIM-PHE, c) CAR-PHE, d) IND-PHE, e) CEL-PHE, f) FUR-PHE, g) NIT-PHE, h) MEB-PHE at equimolar ratios. The black arrows indicate a Tg.

For further analysis, only the co-amorphous drug-PHE mixtures at equimolar molar ratios were taken into consideration. The drugs were chosen and classified into “high-Tg”, “medium-Tg” and “low-Tg” based on the Tg of the pure amorphous drug itself. Each co-amorphous sample exhibited a higher Tg compared to the individual amorphous drugs as shown in Figure 7. A correlation can be seen among the Tgs of the co-amorphous mixtures and those of pure amorphous drugs. As expected and seen in previous studies, the Tgs of co-amorphous samples are higher than the Tgs of the individual amorphous drugs, which is linked to the added excipient, PHE (Strachan *et al.*, 2015). As an example, pure amorphous MEB has the highest Tg in the amorphous drug-group and the Tg of MEB-PHE is the highest Tg in the co-amorphous drug-AA group, etc.

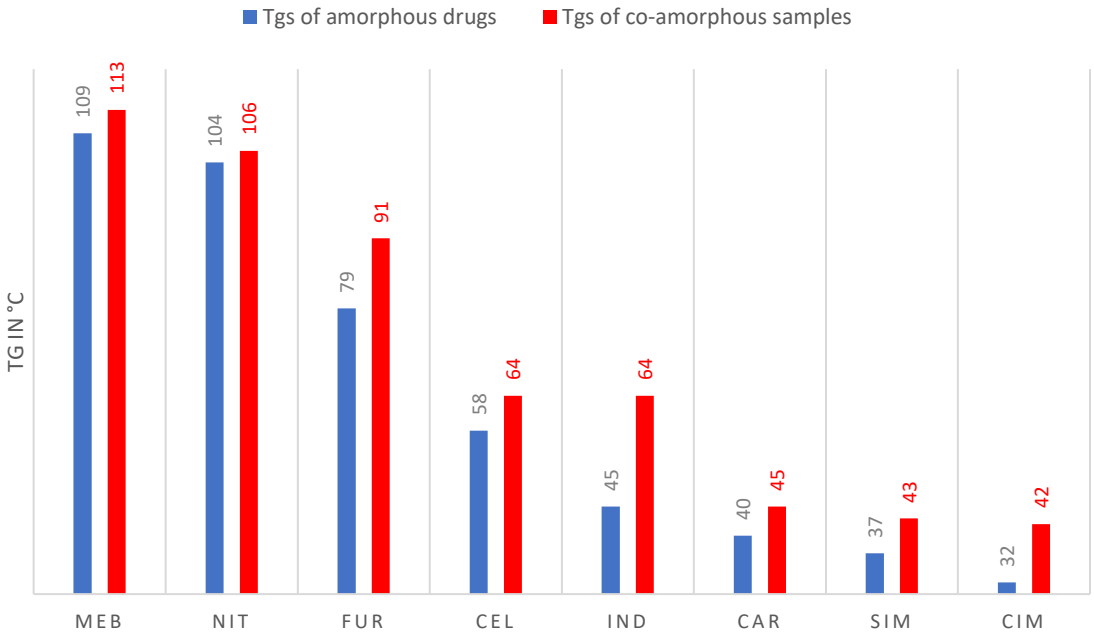


Figure 7. This bar chart shows the correlation of Tgs of amorphous drugs (in blue) and Tgs of co-amorphous drug-PHE samples (in red), from highest to lowest.

An interesting observation is, that CEL-PHE and IND-PHE have the same Tg (64°C), but the pure drugs differ in their Tgs. Amorphous IND has a Tg at 45°C, and amorphous CEL has a Tg at 58°C. A reason for this difference could be the degrees of interactions between the drugs and the AA in both co-amorphous samples. As an indicator for interactions, the Tg values of the calculated hypothetical Tg of PHE have been evaluated with the help of the Fox equation which will be discussed later in the thesis. Other noticeable results are the Tgs of the following binary mixtures: CAR-PHE, SIM-PHE and CIM-PHE. SIM and

CIM are grouped as “low-Tg” drugs, whereas CAR is grouped as a “medium-Tg” drug, yet they show, in combination with PHE at equimolar ratios, similar Tgs. Based on the hypotheses, higher Tg values and stronger molecular interactions within the co-amorphous sample stabilize the co-amorphous so it does not recrystallize too fast. The similar Tg values of CAR-PHE, of SIM-PHE and of CIM-PHE might indicate that their stability at defined storage conditions as well as the degree of molecular interactions regarding the calculated hypothetical Tg of PHE could be comparable.

3.3. Investigation of molecular interactions by FTIR

Vibrational spectroscopy, a set of non-destructive methods, examines solid state properties of materials on the molecular level of the compound (Heinz *et al.*, 2009). Changes in the molecular environment, such as hydrogen bonding and π-π interactions due to solid state interactions can be detected as a result of altered vibrations of functional groups which are involved in these interactions. (Löbmann *et al.*, 2013)

In this study, FTIR spectroscopy was performed to investigate the molecular interactions between the freshly ball-milled drugs (8 in total) and PHE at an equimolar ratio. Ideally, one would compare the IR spectra of the co-amorphous mixtures with reference spectra of their corresponding amorphous as well as crystalline components, in order to verify molecular interactions. However, as crystalline PHE could not be converted into its amorphous state, it was not possible to measure a FTIR spectrum of the amorphous AA. Therefore, the FTIR spectrum of the crystalline AA was used for further analysis in this study. The spectral regions between 800-1800 cm^{-1} and 2300-3500 cm^{-1} were chosen for analysis. They contain information that clearly show changes in the aromatic ring systems (1100–1650 cm^{-1}), hydrogen bonded carboxylic acids (1700 cm^{-1}), amines and hydroxyl group vibrations (3000-3500 cm^{-1}) of the samples (Karagianni *et al.*, 2018).

The main interest in the current study was to investigate the possible formation of molecular interactions between PHE and the respective drug in the co-amorphous systems and whether those interactions are the main reason for the resulting sample stability. Figure 8 shows the spectra of IND-PHE as an example. The results for all investigated samples are shown in section 6.4. “FTIR DATA”.

On first notice the FTIR spectra of crystalline and amorphous IND show, as expected, evident changes in the intensity of their bands. It has been found that, amorphous drugs exhibit slightly broader bands with less intensity (e.g. vibration at 1222 cm^{-1} in crystalline and co-amorphous spectrum) and additional band shifts in comparison to the corresponding crystalline spectra, which refers to a broader range of molecular bonding arrangements within the amorphous form, compared to the crystalline form (Löbmann *et al.*, 2013). Hydrogen bonding is a very frequent form of drug-excipient interactions and generally formed between amine groups (hydrogen donors), carboxylic groups (hydrogen acceptors) and hydroxyl groups (hydrogen acceptors and donors) (Lin *et al.*, 2018).

Changes in the aromatic region (1100 cm^{-1} - 1650 cm^{-1}) point towards π–π interactions in amorphous IND and co-amorphous IND-PHE. The vibrations at 1688 cm^{-1} and 1710 cm^{-1} in the crystalline form belong to the free carboxylic group of the drug and they are shifted to 1677 cm^{-1} and 1705 cm^{-1} in the amorphous form, and to 1680 cm^{-1} and 1710 cm^{-1} in the co-amorphous form with less intensity. Upon closer inspection, one can notice that the vibrations of the co-amorphous form in the region of 800-1800 cm^{-1} closer resemble those of the drug than the AA. In contrast, the region of 2300-3500 cm^{-1} reveals some evident changes when comparing all four spectra. It is as if the bands of both crystalline compounds disappear, merge together and show some, yet very weak vibrations in the co-amorphous sample at ca. 2840 cm^{-1}. It can thus be assumed that the amino group of the AA interacts with the free carboxylic group of IND via hydrogen bonds.

The tables 4a and 4b below list a short analysis of FTIR spectra of all tested samples. For the evaluations the spectra of crystalline drugs and their co-amorphous equivalents were compared. The tables contain information on how the vibrations of crystalline drugs have changed in regard to the co-amorphous samples, and which functional groups took part in forming molecular interactions with the AA. This gives a first clue of the presence of molecular interactions between each drug and PHE, which are important for the co-amorphous samples to stay stable.

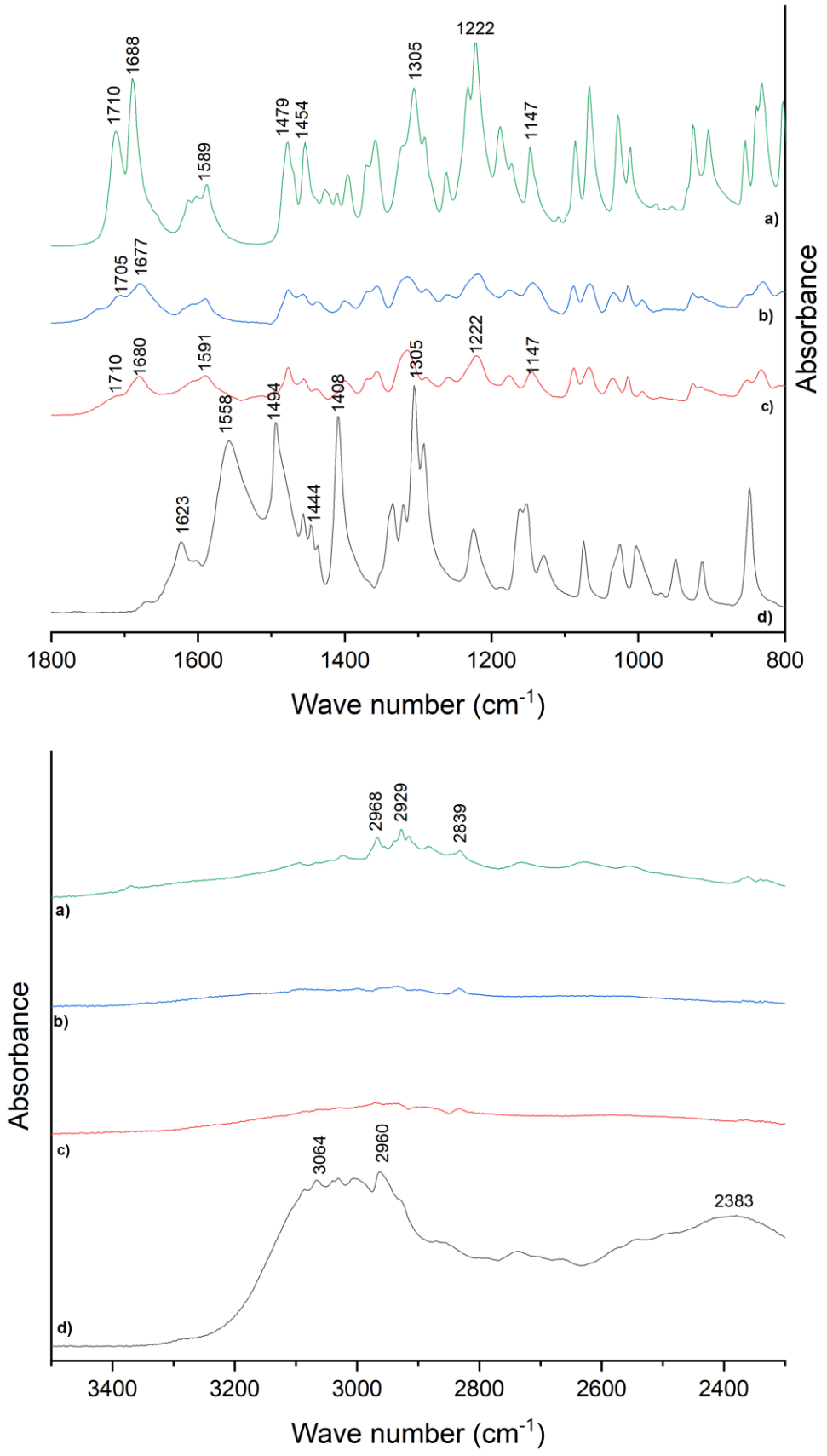


Figure 8. FTIR spectra of a) crystalline IND, b) amorphous IND, c) co-amorphous IND-PHE, d) crystalline PHE in the regions of 3500-2300 cm^{-1} and 1800-800 cm^{-1}.

Table 4a. FTIR spectra evaluation of CAR-PHE, CEL-PHE, CIM-PHE and FUR-PHE

Co-amorphous samples	Vibrations of crystalline drugs	Vibrations od co-amorphous samples	Information	Interaction between drug and PHE
CAR-PHE	• 1097 cm^{-1} – 1623 cm^{-1} • 2835 cm^{-1} – 3344 cm^{-1}	• Merged together and less intensive • Merged together and less intensive	• Aromatic region • Amines and hydroxylic groups	yes
CEL-PHE	• 1100 cm^{-1} – 1623 cm^{-1} • 2980 cm^{-1} – 3332 cm^{-1}	• Merged together and less intensive • Merged together, no visible vibrations	• Aromatic region • Amines, S=O, N=N	yes
CIM-PHE	• 1201 cm^{-1} – 1623 cm^{-1} • 2920 cm^{-1} – 3218 cm^{-1}	• Merged together and less intensive • Merged together and less intensive	• Aromatic region • Amines, nitrile	yes
FUR-PHE	• 1140 cm-^{1} – 1623 cm-^{1} • 1670 cm^{-1} • 2980 cm^{-1} • 3278 cm^{-1} – 3400 cm^{-1}	• Merged together and less intensive • Shifted to 1675 cm^{-1} • Weaker vibration • Merged together and less intensive	• Aromatic region • COOH • Furan • Amines, -O-	yes

Table 4b. FTIR spectra evaluation of IND-PHE, MEB-PHE, NIT-PHE and SIM-PHE

Co-amorphous samples	Vibrations of crystalline drugs in the regions of:	Change in vibrations of co-amorphous samples	Information	Interaction between drug and PHE
IND-PHE	• 1147 cm^{-1} – 1623 cm^{-1} • 1688 cm^{-1} – 1710 cm^{-1} • 2839 cm^{-1} – 2968 cm^{-1}	• Merged together and less intensive • Shifted to 1680 cm^{-1} and 1710cm^{-1} • Merged together and less intensive	• Aromatic region • Free COOH, C=O • Free OCH3, amid stretching	yes
MEB-PHE	• 1091 cm^{-1} – 1623 cm^{-1} • 1730 cm^{-1} • 2945 cm^{-1} • 3367 cm^{-1}	• Merged together and less intensive • Shifted to 1728 cm^{-1} • Less intensity of vibration • Shifted to 3392 cm^{-1} and decreased in intensity	• Aromatic region • Ketone • Free OCH3 • Amino and hydroxyl group stretching	yes
NIT-PHE	• 1207cm^{-1} – 1623 cm^{-1} • 1728 cm^{-1} & 1779 cm^{-1} • 3016 cm^{-1} – 3280 cm^{-1}	• Merged together and less intensive • Shifted to 1726 cm^{-1} & 1785 cm^{-1} • Merged together and less intensive	• Aromatic region, C=C • C=O stretching • Amines, N=O, NH	yes
SIM-PHE	• 1162 cm^{-1} – 1623 cm^{-1} • 1695 cm^{-1} • 2870 cm^{-1} – 3008 cm^{-1}	• Merged together and less intensive • Shifted to 1716 cm^{-1} • Merged together and less intensive	• Aromatic region • C=O stretching • OH vibration	yes

3.4. Hypothetical Tg of PHE – indication for strength of molecular interactions

The Gordon-Taylor equation (GT) is one possible way of estimating a theoretical Tg of co-amorphous mixtures if the Tgs of both amorphous compounds can be measured (Skrdla *et al.*, 2017). Two different methods, with the aim to convert the crystalline state of PHE into its amorphous form, proved to be insufficient, and consequently it was not possible to measure an experimental Tg of PHE. GT necessitates the use of the compounds´ densities, which was not taken into consideration for this study. An easier way to estimate a Tg of a co-amorphous compound is the so-called Fox equation, which does not require the densities of both samples (Kalogeras 2016):

$$\frac{1}{Tg_{1,2}} = \frac{W_1}{Tg_1} + \frac{W_2}{Tg_2}$$

Where W_1 and W_2 are the weight fractions and Tg_1 and Tg_2 the experimentally measured Tgs of substance 1 and 2, respectively. The attempt for this study was to determine the Tg of PHE by rearranging the Fox equation with respect to the Tg_{AA} and calculate a hypothetical Tg of PHE for each equimolar drug-PHE co-amorphous sample.

$$T_{gAA} = \frac{\left[\left(\frac{1}{T_{g1,2}}\right) - \left(\frac{W_D}{T_{gD}}\right)\right]}{W_{AA}}$$

Tg_{AA} is the calculated, hypothetical Tg of PHE, W_D and W_{AA} are the weight fractions of the drug and PHE, respectively, Tg_D is the measured Tg of the amorphous drug, and $Tg_{1,2}$ is the experimentally determined Tg of the co-amorphous sample. Table 5 shows the calculated hypothetical Tgs for each co-amorphous mixture. If there were no interactions between the drug and PHE, then the calculated value for Tg_{AA} would be the same. The different Tg_{AA} values suggest, that some co-amorphous samples do have stronger interaction than others, and based on our hypotheses, samples with stronger interactions should show better stability results.

Table 5. Tgs of the amorphous drugs and their equivalent co-amorphous mixtures. With the help of the Fox equation it was possible to calculate the hypothetical Tg of PHE for every equimolar ratio sample.

	Pure Drugs	Drug-AA (co-amorphous, equimolar)	hypothetical Tg of PHE (Tg_{AA})
high-Tg	Mebendazole 109°C / 382 K	MEB-PHE 113°C / 386 K	120,3°C / 393,3 K
	Nitrofurantoin 104°C / 377K	NIT-PHE 106°C / 379 K	108,9°C / 381,9 K
	Furosemide 79°C / 352 K	FUR-PHE 91°C / 364 K	117°C / 390 K
medium-Tg	Celecoxib 58°C / 331 K	CEL-PHE 64°C / 337K	78,7°C / 351,7 K
	Indomethacin 45°C / 318 K	IND-PHE 64°C / 337 K	114°C / 387 K
	Carvedilol 40°C / 311 K	CAR-PHE 45°C / 316 K	56°C / 329 K
low-Tg	Simvastatin 37°C / 310 K	SIM-PHE 43°C / 316 K	59,3°C / 332,3 K
	Cimetidine 32°C / 305 K	CIM-PHE 42°C / 315 K	58,6°C / 331,6 K

Table 6 shows the calculated hypothetical Tg of PHE as an indicator for interactions for each binary co-amorphous form of drug-PHE, from highest to lowest. Referring to our hypothesis, we wanted to find out if higher values for the calculated hypothetical Tg of PHE lead to stronger interactions with the drug, resulting in a more stable co-amorphous system. For example, according to the calculations, co-amorphous MEB-PHE has very strong interactions compared to co-amorphous CAR-PHE, because the calculated hypothetical Tg of PHE is much higher for MEB-PHE than for CAR-PHE.

Table 6. Tgs of co-amorphous drug-PHE samples as well as the calculated hypothetical Tg of PHE for each binary sample. All hypothetical Tgs of PHE act as indicators regarding the stability of the respective drug-PHE sample.

Drug-PHE (co-amorphous, equimolar)	Hypothetical Tg of PHE
MEB-PHE 113°C / 386 K	120,3°C / 393,3 K
FUR-PHE 91°C / 364 K	117°C / 390 K
IND-PHE 64°C / 337K	114°C / 387 K
NIT-PHE 106°C / 379 K	108,9°C / 381,9 K
CEL-PHE 64°C / 337K	78,7°C / 351,7 K
SIM-PHE 43°C / 316 K	59,3°C / 332,3 K
CIM-PHE 42°C / 315 K	58,6°C / 331,6 K
CAR-PHE 45°C / 316 K	56°C / 329 K

It is important to note that this is only a calculated hypothetical Tg of PHE, not the actual one. Four combinations show high calculated hypothetical Tgs above 100°C, one shows a medium and three show a low calculated hypothetical Tgs of PHE. This indicates that the calculated hypothetical high Tgs for PHE must come from more and / or stronger interactions with the respective drug of each combination and that MEB-PHE, FUR-PHE, IND-PHE and NIT-PHE should, according to our hypothesis, stay stable for a longer period than the other four samples. IND and CEL have been compared to each other in Section 3.2 “Determination of thermal properties by DSC” in terms of their different Tgs as amorphous drugs but same Tgs as co-amorphous forms. After calculating the hypothetical Tg of PHE for IND-PHE at 114°C and for CEL-PHE at 78,7°C, it can be assumed that, even though both co-amorphous forms are in the same group and have the same Tg in their co-amorphous state, the degree of interaction between IND and PHE in the co-amorphous form might be stronger than for CEL-PHE which may result in different stability outcomes.

3.5. Storage stability of “controlled” and “uncontrolled binary samples”

3.5.1. “Controlled binary samples”

The following bar charts show the stability of the co-amorphous samples at equimolar ratios and the temperatures under which they were stored. After determining the Tg for each binary form, the storage temperatures were selected as follows:

- the samples were stored below the lowest Tg of each group, but still close enough to that Tg to stress the systems. To clarify, FUR-PHE has the lowest Tg of the “high-Tg” co-amorphous samples at 91°C and therefore FUR-PHE, NIT-PHE, MEB-PHE were stored in the oven at 80°C. In our hypotheses we state that samples with higher Tgs and/or stronger molecular interactions are more stable than those with lower Tgs and/or weaker molecular interactions
- all equimolar samples were stored at RT as well
- all equimolar samples were stored in desiccators under dry conditions

In the first month of storage, the samples were analysed on a regular basis using XRPD to monitor the onset of recrystallization of the compounds. If there were peaks originating from only one of the substances, drug or PHE, the stability test was continued. As soon as both substances started to recrystallise, the stability measurement for that co-amorphous mixture was terminated. The duration of the entire stability assessment lasted 70 days.

“Low-Tg” co-amorphous samples at RT (Figure 9):

Only two out five drugs, that were chosen for this group, turned co-amorphous with PHE and those samples were stored only at RT. For both, CIM-PHE and SIM-PHE, the AA was the first component that started to recrystallise and showed peaks in the XRPD diffractograms after six days for SIM-PHE and after 13 days for CIM-PHE. No peaks belonging to the crystalline drug were found until the termination of the study.

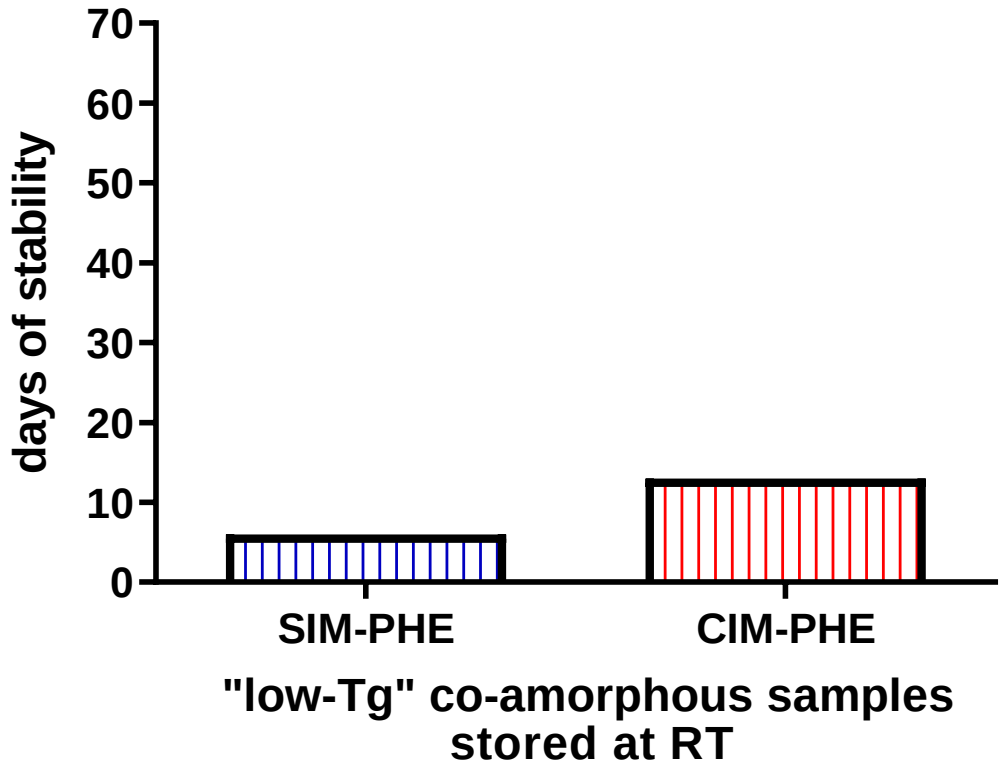
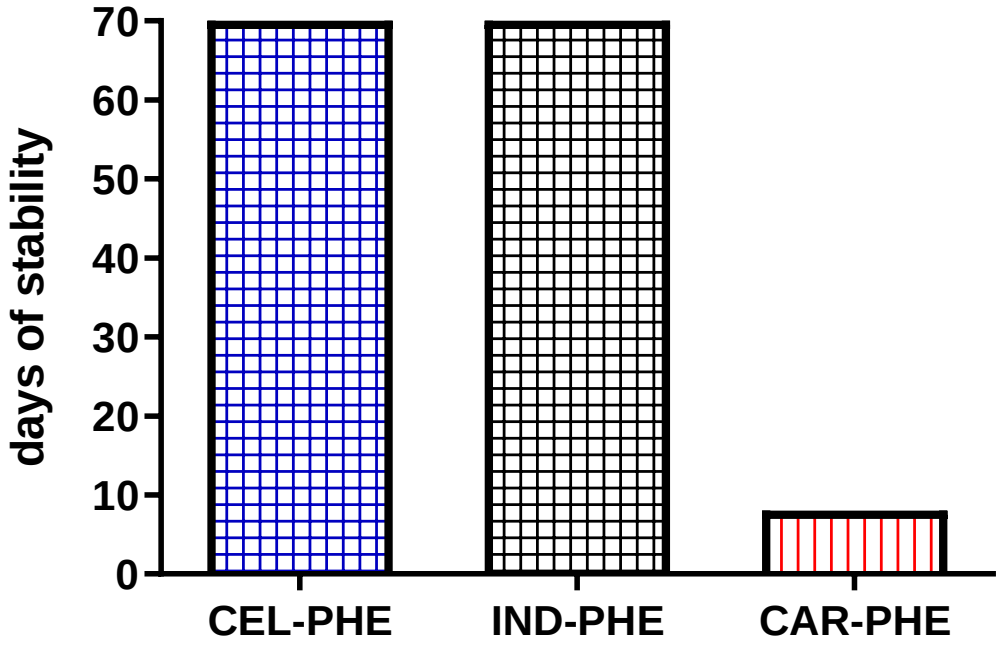
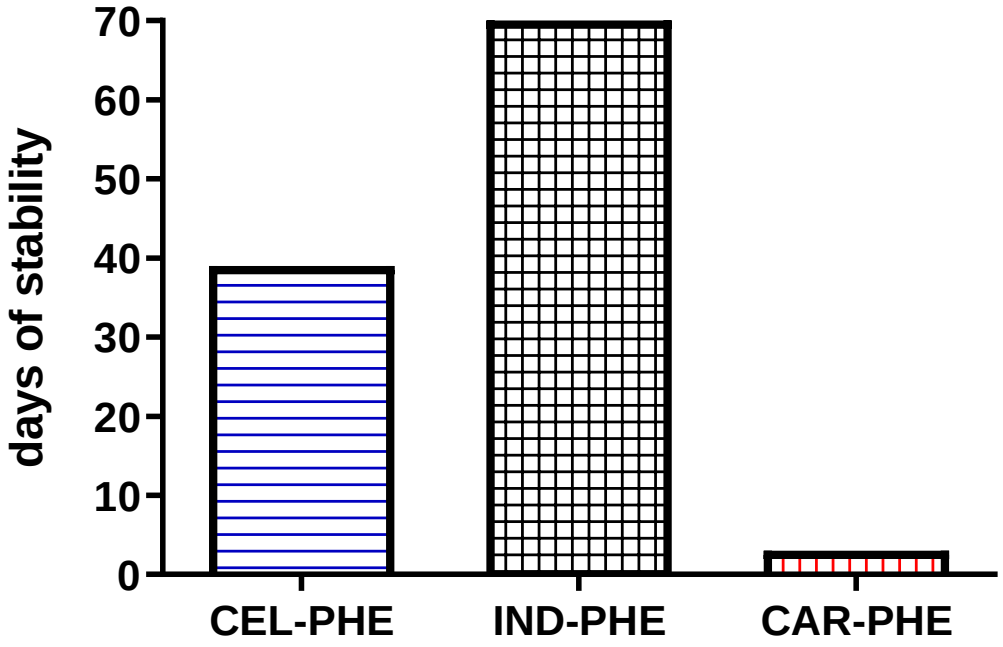


Figure 9. Stability results of “low-Tg” co-amorphous samples, SIM-PHE and CIM-PHE, stored at RT.

“Medium-Tg” co-amorphous samples at RT (Figure 10a) and 40°C (Figure 10b):

The samples were stored under two conditions: at RT, at around 22°C, and at 40°C, as CAR-PHE had the lowest Tg (45°C) in this group. CEL-PHE and IND-PHE stayed stable at RT from starting the stability test until the end of the study (70 days). CAR-PHE at RT showed a similar pattern to the two “low-Tg” co-amorphous samples as PHE was the first compound that started to recrystallise after only eight days, but CAR stayed stable in that combination. This could be linked to the similar Tgs of the “low-Tg” co-amorphous samples but also to the similar values of the calculated hypothetical Tgs of PHE indicating a low degree of molecular interactions. As in its RT storage outcome, the XRPD diffractogram of CAR-PHE stored at 40°C revealed an early onset of recrystallisation; after only three days of storage PHE started to recrystallise, but not CAR. After 39 days of storage at 40°C the diffractogram of CEL-PHE showed peaks of crystalline CEL, but not of PHE. IND-PHE stored at 40°C was the only co-amorphous sample out of the three, that stayed stable and showed no signs of recrystallisation. This finding is in line with previous studies, that confirm, that IND in combination with an AA as a co-amorphous form, can stay stable for up to 6 months at 40°C (Löbmann *et al.*, 2013). Even though IND-PHE and CEL-PHE have the same Tg at 64°C their different stability outcome can be linked to the different hypothetical Tg values of PHE, and therefore to different strengths of interactions.





10a+10b. Stability results of "medium-Tg" co-amorphous samples, CEL-PHE, IND-PHE and CAR-PHE, stored at RT and at 40°C.

“High-Tg” co-amorphous samples stored at RT (Figure 11a) and at 80°C (Figure 11b): These co-amorphous mixtures stayed stable at RT for the entire duration of the storage experiments (70 days). At 80°C however, some unexpected events occurred. Referring to our hypotheses, we claimed the height of Tg of a sample as well as the molecular interactions within the same sample play an important role when defining the sample´s stability and that therefore co-amorphous samples with higher Tgs should stay stable for a longer period than those with lower Tgs, when kept at the same temperature and storage conditions. Co-amorphous NIT-PHE and MEB-PHE have Tgs at 106°C and 113°C, the Tg of co-amorphous FUR-PHE only reaches up to 91°C. Our storage temperature was 80°C, below, yet close to FUR-PHE´s Tg to stress the system and therefore we expected to see some significant changes in its XRPD diffractogram early on in the storage study. But surprisingly this was not the case. Within 24 hours of storage at 80°C it was co-amorphous NIT-PHE that recrystallized - both drug and PHE showed peaks in the XRPD diffractogram. For MEB-PHE the AA was the first component to show peaks, also within 24 hours, whereas the drug recrystallized after six days. For co-amorphous FUR-PHE it took ten days for PHE to recrystallize and another ten days for the drug to show crystallinity in its XRPD diffractograms.

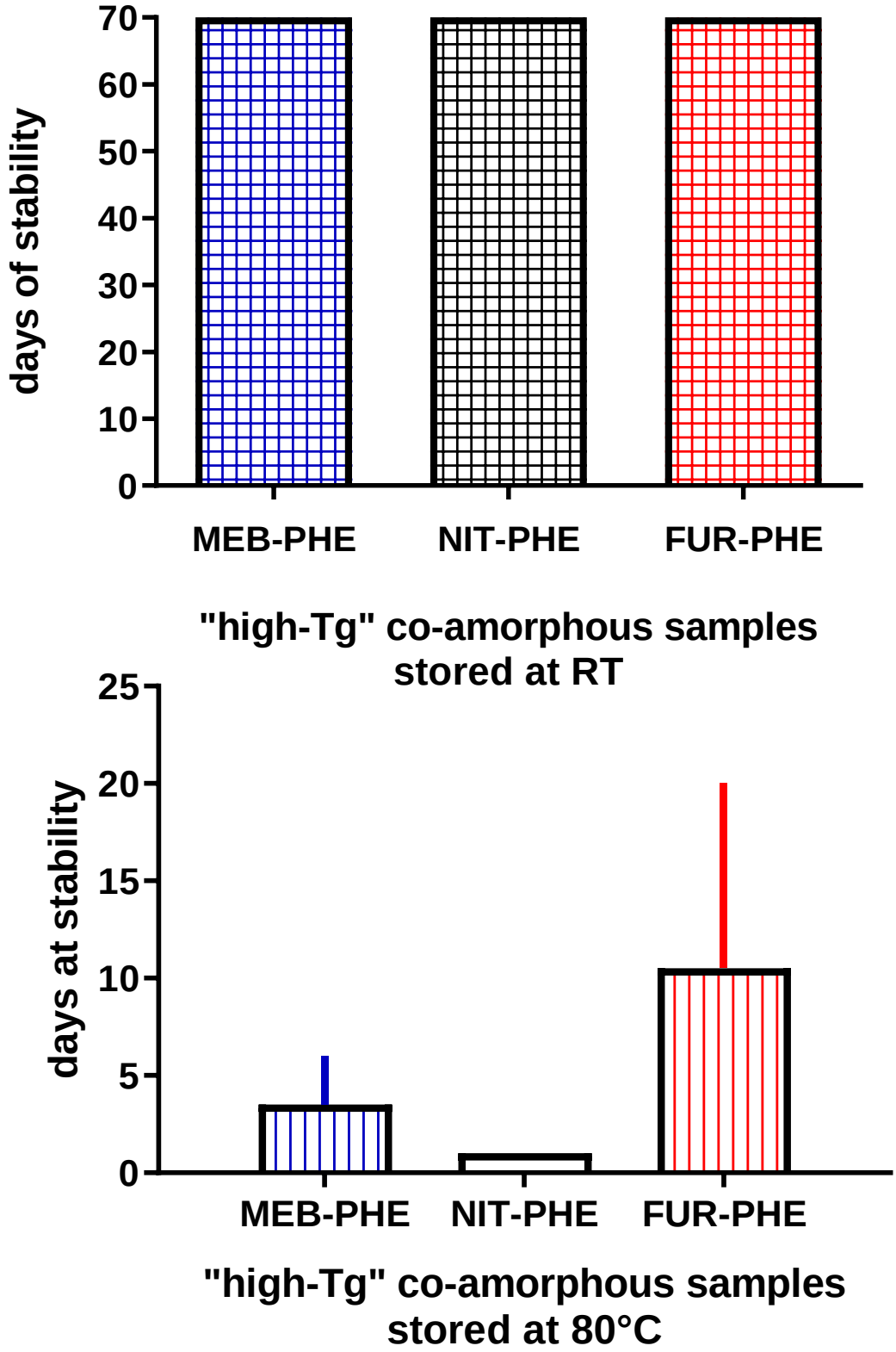


Figure 11a+11b: Stability results of "high-Tg" co-amorphous samples, MEB-PHE, NIT-PHE and FUR-PHE, stored at RT, on the left and at 80°C, on the right.

Interestingly, among the eight co-amorphous mixtures with PHE as the common denominator, the samples with the highest Tg values (MEB-PHE and NIT-PHE) were not the most stable ones and both components, drug and the AA, recrystallized after a very short storage time. This contrasts with the other samples, where only one of the two components, drug or AA, showed peaks and the other one stayed stable. Figure 12 is an example of how the XRPD diffractograms were analysed. It shows the diffractograms of crystalline NIT and PHE, the freshly ball-milled co-amorphous NIT-PHE, stable NIT-PHE at RT after termination of the study and recrystallized NIT-PHE at 80°C after only 24 hours. One easy way to spot recrystallization in the co-amorphous form is by overlapping the spectra and search for all the peaks in the recrystallized co-amorphous form, that can be assigned to the crystalline drug and/or AA

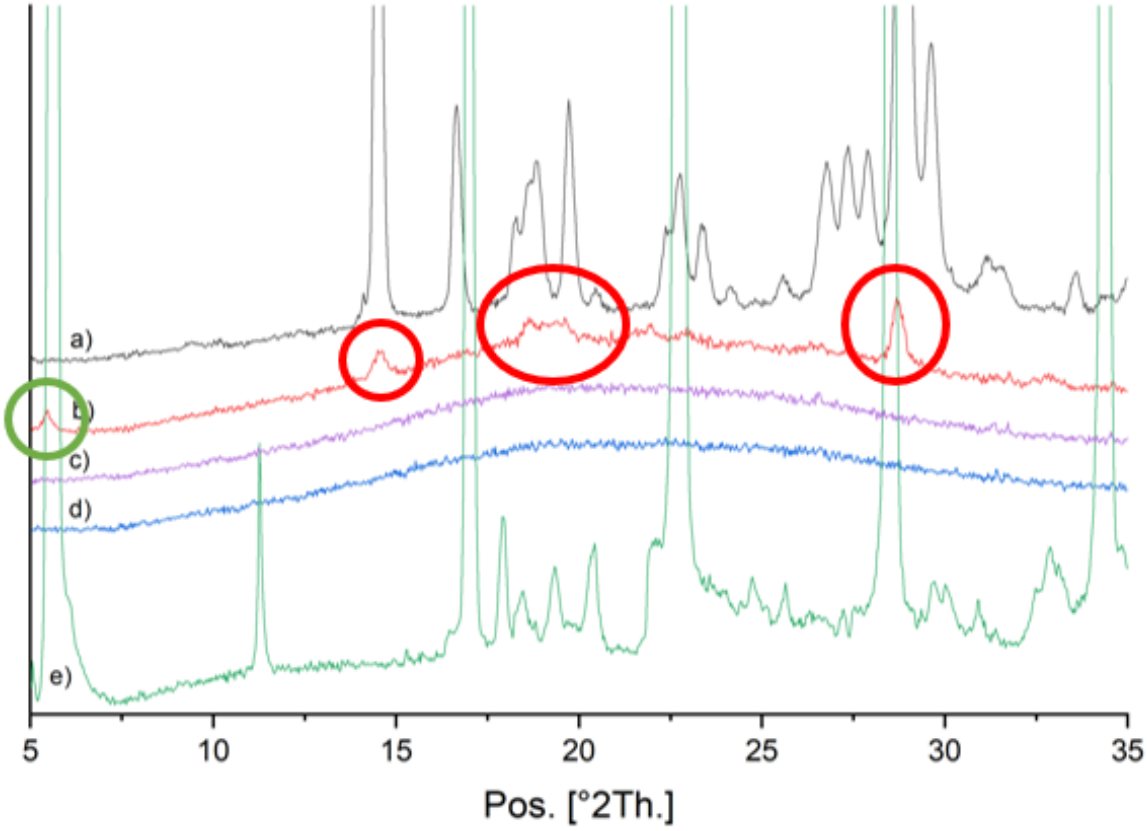


Figure 12: XRPD diffractograms of a) crystalline NIT, b) recrystallised NIT-PHE at 80°C after 24 hours, c) stable NIT-PHE at RT after 70 days, d) freshly ball-milled co-amorphous NIT-PHE, e) crystalline PHE. The circles point out peaks from the recrystallized co-amorphous NIT-PHE that belong to the AA, shown in green, and the drug, shown in red.

This stability outcome reveals that the Tg value is not the only responsible factor for the stability of amorphous systems, but the molecular interactions must also be taken into consideration. The calculated hypothetical Tg of PHE is used as mentioned as an indicator for the extent or strength interactions. The calculations for MEB-PHE show that the system must have strong interactions as the calculated hypothetical Tg of PHE is at 120°C, yet the AA is starting to crystallize within 24 hours and the drug only after six days, when on stability at 80°C (Table 7). This means that neither the strength of interactions nor the absolute position of the Tg of the co-amorphous sample alone can be used as a sole indicator for the stability and onset of recrystallisation.

Table 7. Stability ranking of the samples that were stored at "controlled conditions". It shows their storage temperature, the total time of recrystallization until the study was terminated and which component recrystallized. When kept at RT only two samples out of fourteen recrystallized; when kept at higher temperatures only one out of fourteen did not recrystallize.

	Drug-PHE (co-amorphous, equimolar)	**Total time of recrystallization until the end of study**	**Recrystallized component(s)**
1.	NIT-PHE (80°C stability)	24 hours	NIT and PHE
2.	CAR-PHE (40°C stability)	3 days	PHE
3.	MEB-PHE (80°C stability)	6 days	MEB and PHE
4.	SIM-PHE (RT stability)	6 days	PHE
5.	CAR-PHE (RT stability)	8 days	PHE
6.	CIM-PHE (RT stability)	13 days	PHE
7.	FUR-PHE (80°C stability)	20 days	FUR and PHE
8.	CEL-PHE (40°C stability)	39 days	CEL
9.	IND-PHE (40°C stability)	stable	-
10.	MEB-PHE (RT stability)	stable	-
11.	NIT-PHE (RT stability)	stable	-
12.	FUR-PHE (RT stability)	stable	-
13.	CEL-PHE (RT stability)	stable	-
14.	IND-PHE (RT stability)	stable	-

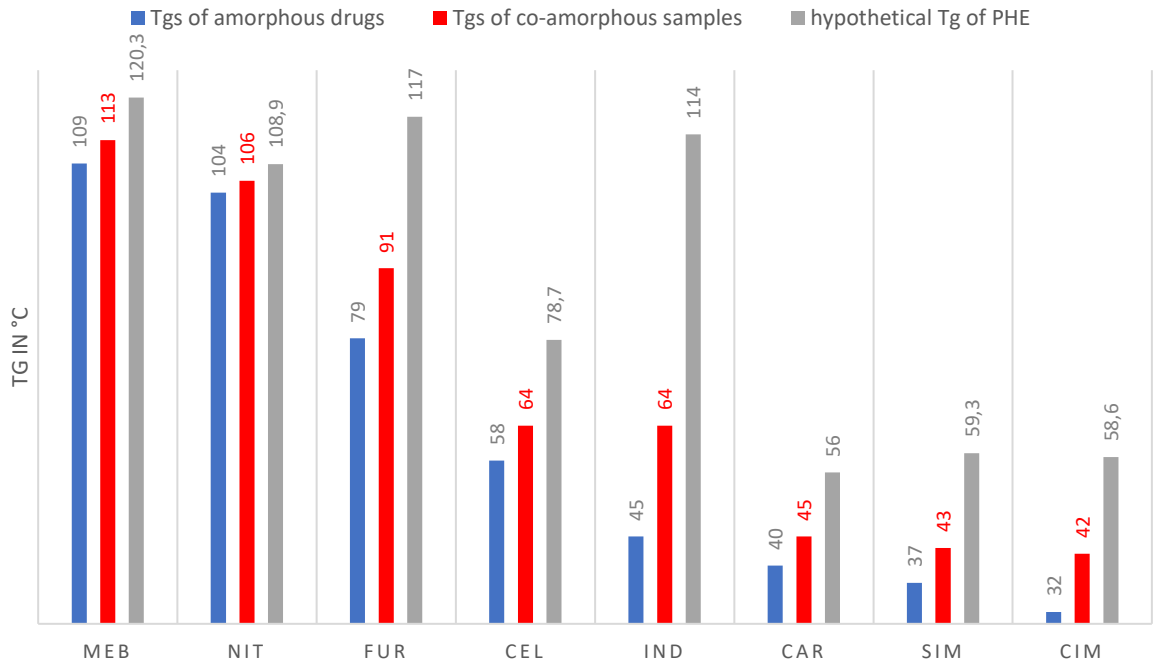


Figure 13 This graph shows three different sets of Tgs: Tgs of amorphous drugs shown in blue, Tgs of co-amorphous drug-PHE samples shown in red and hypothetical Tgs of PHE shown in grey.

Figure 13 is similar to figure 7, but this time includes the hypothetical Tg values of PHE. This was another approach to evaluate, why the stability results of some the co-amorphous samples at "controlled conditions" turned out different than expected. Besides examining the Tg values and molecular interactions, a closer look is taken at the distance between the Tgs of co-amorphous samples and the hypothetical Tgs of PHE in order to correlate that distance to the stability results. The shortest distance between the Tg of a co-amorphous sample and its hypothetical Tg of PHE is 2,9°C for NIT-PHE. NIT-PHE was also the first co-amorphous sample (stored at 80°C) to fully recrystallize within 24 hours. The only co-amorphous sample that stayed stable throughout the entire study was IND-PHE, and with no surprise has the furthest distance between co-amorphous Tg and hypothetical Tg of PHE.

Correlation of the distance between co-amorphous Tgs and hypothetical Tgs of PHE is showing to be a possible way to estimate the stability of co-amorphous samples. However, it is important to mention that it does not take into account the different storage temperatures and therefore the different time of recrystallization for the same sample at different storage conditions.

3.5.2. “Uncontrolled binary samples”

The second set samples (23 in total) has been stored at what is defined in this study as “uncontrolled conditions”. Those samples were kept in a storage box at RT and include pure amorphous drugs, co-amorphous 25% drug – 75% PHE samples and co-amorphous 75% drug – 25% PHE samples, at molar ratios. Initially those samples were prepared to estimate the Tg of pure PHE. Jensen and co-workers claimed that by evaluating the Tgs of co-amorphous samples at different ratios, the Tg of one amorphous compound can be estimated, even though it could not be produced experimentally as a neat amorphous form (Jensen *et al.*, 2016). This approach proved to be not feasible in this study, as, although PHE could not be converted into an amorphous form on its own, the calculated hypothetical Tg resulted in a different value for each drug-PHE combination (see Table 5). Nevertheless, the samples were kept on storage at “uncontrolled conditions” and their stability was tracked after one and two months, using XRPD. The purpose of this attempt was to see if co-amorphous mixtures of drug-AA at different molar ratios would be more useful than equimolar ratios, regarding the time of stability. Results of this stability study are shown in Tables 8a and 8b.

Table 8a. Stability results of 24 samples after one month of storage at "uncontrolled conditions". Nine samples of co-amorphous mixtures and amorphous drugs showed no sign of crystallinity. Fourteen samples revealed peaks in their XRPD diffractograms indicating recrystallization of the drug and / or AA and one sample could not be made co-amorphous and was therefore not used for the Tg measurements by DSC and later for the stability analysis.

		25% drug – 75% PHE	75% drug – 25% PHE	100% drug
High Tg co-amorphous	MEB-PHE	AA recrystallized	stable	stable
	NIT-PHE	stable	stable	recrystallized
	FUR-PHE	AA recrystallized	stable	recrystallized
Medium Tg co-amorphous	CEL-PHE	drug recrystallized	stable	recrystallized
	IND-PHE	AA recrystallized	stable	recrystallized
	CAR-PHE	drug recrystallized	drug recrystallized	recrystallized
Low Tg co-amorphous	SIM-PHE	Could not be made amorphous	AA recrystallized	stable
	CIM-PHE	AA recrystallized	stable	recrystallized

Table 8b. **S**tability results of 23 samples after two months of storage at "uncontrolled conditions". Only seven samples, six co-amorphous mixtures and one amorphous drug, showed no sign of crystallinity after two months. The XRPD diffractograms of two samples, amorphous MEB and co-amorphous NIT-PHE (25%-75% molar ratio), that were stable after one month, detected onsets of recrystallization of the drug and / or AA after the second month of stability analysis.

		25% drug – 75% PHE	75% drug – 25% PHE	100% drug
High Tg co-amorphaus	MEB-PHE	AA recrystallized	stable	recrystallized
	NIT-PHE	AA recrystallized	stable	recrystallized
	FUR-PHE	AA recrystallized	stable	recrystallized
Medium Tg co-amorphous	CEL-PHE	AA and drug recrystallized	stable	recrystallized
	IND-PHE	AA recrystallized	stable	recrystallized
	CAR-PHE	AA and drug recrystallized	drug recrystallized	recrystallized
Low Tg co-amorphous	SIM-PHE	Could not be made amorphous	AA and drug recrystallized	stable
	CIM-PHE	AA and drug recrystallized	stable	recrystallized

After two months of storage all samples containing 25% drug and 75% PHE recrystallized, except for SIM-PHE which could not be converted into a co-amorphous form at all. As expected, and shown in several studies before, all pure amorphous drugs showed peaks after one month, except for MEB and SIM (Löbmann *et al.*, 2013; Karagianni *et al.*, 2018). After two months of storage MEB started to convert back into its crystalline state, and only the diffractogram of SIM remained without noticeable peaks. Apart from CAR-PHE and SIM-PHE, all other samples containing 75% drug and 25% PHE stayed stable and showed no signs of recrystallization throughout the two months of stability study. Comparing the stability results of the samples that were stored at "uncontrolled conditions" with those that were kept at "controlled conditions", it can be stated that the choice of preparing samples at equimolar ratios must not always be ideal. For instance, CIM-PHE at equimolar ratio started to show peaks that belonged to PHE after only 13 days. On the other hand, CIM-PHE at the 75% - 25% molar ratio was still fully amorphous after two months. Moreover, all three "high-Tg" co-amorphous forms and two of the "medium-Tg" co-amorphous forms at molar ratios of 75% drug – 25% PHE showed no sign of recrystallization. This implies that more drug and less AA might need to be used, to create stable co-amorphous compounds.

4. Conclusion and future work

This study focused on the use of one AA as an excipient for eight different drugs to stabilize the amorphous form of those drugs as co-amorphous systems, and to examine the onset of recrystallization (and thus physical stability) of those co-amorphous mixtures upon different storage temperatures. Ball-milling proved to be a successful preparation technique to convert almost all investigated drug-AA mixtures into their respective co-amorphous form; the AA PHE showed very good co-formability as a co-former for eight out of eleven tested drugs (72%). The binary mixtures were spilt into three groups ("low-Tg", "medium-Tg" and "high-Tg") in relation to the Tg of the neat amorphous drug in each binary co-amorphous form and stored under "controlled conditions" at RT and at temperatures below the lowest Tg in each group. The calculated hypothetical Tg value of PHE was used as an indicator for the extent or strength of interactions in the co-amorphous form between the drug and the AA. The higher the calculated hypothetical Tg value of PHE for each binary compound, the more interactions are assumed to occur with the drug and therefore the more stable that binary compound should be. Surprisingly, the order of increasing Tg values of the "controlled samples" did not totally go hand in hand with the order of their physical stability. The co-amorphous forms of SIM-PHE and CIM-PHE (at RT) and CAR-PHE (at RT and 40°C) showed an early onset of recrystallisation after three to thirteen days with peaks belonging to the AA. Those three co-amorphous samples had the lowest calculated hypothetical Tgs values for PHE, indicating very low interaction between the drug and the AA. For the co-amorphous form of CEL-PHE it was the drug that started to recrystallise first. The calculations for all three "high-Tg" co-amorphous forms, MEB-PHE, NIT-PHE and FUR-PHE, suggest strong interactions between PHE and each drug but in fact all three recrystallized in a timeframe of 24 hours up to several days when stored at 80°C, but remained stable at RT. Out of all binary compounds only one co-amorphous compound (IND-PHE) with calculated strong interactions and a medium Tg remained stable throughout the whole study and showed superb physical stability at RT and 40°C.

Taking all of that into consideration, the stability outcome reveals that neither the intensity of interactions in co-amorphous binary mixtures at an equimolar molar ratio alone nor their Tgs alone can be taken as a parameter to determine their

physical stability. Amorphous drugs with similar Tgs as well as their co-amorphous forms with similar Tgs show similar hypothetical Tgs values of PHE, suggesting similar strengths of molecular interactions and comparable stability outcomes. On the other hand, co-amorphous forms with the same Tg but different hypothetical Tgs of PHE, differ a lot in their stability outcomes due to different degrees of molecular interactions within the systems.

To get a better understanding of the dependency of solid-state stability, the interactions between drugs and their excipients need a more detailed quantification such as using spectroscopic measurements, for example ssNMR (Schantz *et al.*, 2009; Newman *et al.*, 2017).

There have been some studies suggesting to use AAs with low pKa values as co-formers as they reveal weak molecular interactions when combined with drugs and therefore the different stability outcomes might be a result from different intermolecular interaction strengths in each co-amorphous mixture (Huang *et al.*, 2016). This would present a new way of choosing a suitable co-former. PHE has shown good co-former characteristics with almost all drugs. However about 40% of all produced co-amorphous samples (15 out of 37) revealed an onset of crystallization in their XRPD diffractograms with peaks belonging to PHE, while performing stability measurements. Hence it can be assumed, that PHE is a suitable AA for preparing co-amorphous samples but lacks the ability to keep those systems stable.

One different approach could be to analyse co-amorphous systems that are mixed at individual molar ratios and not just at equimolar ratio. For this, the binary mixtures kept at „uncontrolled conditions" can be taken as examples, as some samples stayed stable at molar ratios consisting of 75% drug and 25% AA, whereas their corresponding equimolar samples at "controlled conditions" did not.

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6. Appendix

6.1. Zusammenfassung (auf Deutsch)

Ungefähr 90% der Arzneistoffe, die sich derzeit in Entwicklung befinden, weisen eine schlechte Wasserlöslichkeit auf, was zu einer geringeren oralen Absorption und somit einer noch geringeren Bioverfügbarkeit führt. Aus diesem Grund hat es sich die pharmazeutische Industrie und Forschung zur Aufgabe gemacht, neue Strategien zur Überwindung des Problems der niedrigen Auflösungsrate und der mangelnden Löslichkeit zu untersuchen und zu erproben. Eine dieser Strategien ist die Co-Amorphisierung, bei der zwei oder mehrere Substanzen aus ihrem kristallinen Zustand in eine co-amorphe Mischung umgewandelt werden und deren Moleküle dadurch keine regelmäßige Anordnung mehr aufweisen. Dies erwies sich als geeigneter Ansatz, neue Arzneizubereitungen herzustellen, die sowohl die Auflösungsrate und Löslichkeit schwer wasserlöslicher Wirkstoffe steigern, als auch die physikalische Stabilität rein amorpher Medikamente während ihrer Lagerung verbessern.

Diese Arbeit beschäftigte sich hauptsächlich mit der Frage, ob die Stabilität co-amorpher binärer Verbindungen äquimolarer Mengen überwiegend von ihrer Glasübergangstemperatur (Tg) oder den molekularen Wechselwirkungen innerhalb der Mischung oder von beiden Faktoren gleichzeitig abhängig ist, während sie bei unterschiedlichen Temperaturen gelagert wurden. Für die experimentelle Durchführung dieser Diplomarbeit wurde eine unpolare Aminosäure (Phenylalanin) als Hilfsstoff ausgewählt, um mit elf verschiedenen Wirkstoffen, die sich in ihren Strukturen und pharmakologischen Eigenschaften unterscheiden, co-amorphe binäre Verbindungen zu bilden.

Im ersten Teil der Arbeit war es das Ziel, co-amorphe Verbindungen herzustellen. Mithilfe von oszillierenden Kugelmühlen wurden für jede Arzneistoff-PHE Verbindung vier Proben hergestellt - amorphe Reinsubstanzen und Mischungen in molaren Verhältnissen von: 25% Wirkstoff - 75% PHE, 50% Wirkstoff - 50% PHE, 75% Wirkstoff - 25% PHE. Die Messungen der Röntgenpulverbeugung (XRPD) und Differentiellen Scanning Kalorimetrie (DSC) ergaben, dass fast alle Mischungen eine diffuse Streuung, auch „Halo“ genannt, in ihren Diffraktogrammen hatten sowie eine einzige Glasübergangstemperatur (Tg) laut DSC-Messungen besaßen, was bedeutet, dass die Mischungen erfolgreich in ihre

co-amorphen Formen überführt werden konnten. Die Messungen der Fourier-Transformations-Infrarotspektroskopie (FTIR) ergaben molekulare Wechselwirkungen, die für die Bildung und Aufrechterhaltung der co-amorphen Zustände verantwortlich sind.

Der zweite Teil der Arbeit beschäftigte sich mit der Beurteilung der Stabilität der co-amorphen Verbindungen. Proben, bestehend aus äquimolaren Mengen Wirkstoff und PHE, wurden ausgehend von ihren Tgs in drei Gruppen aufgeteilt und bei festgelegten Temperaturen („kontrollierte Bedingungen“) gelagert. Um den amorphen Zustand und Beginn einer Rekristallisation zu beobachten, wurden die Proben in regelmäßigen Zeitabständen mit XRPD gemessen. PHE war eine der wenigen Substanzen, deren kristalliner Zustand nicht in den amorphen Zustand überführt werden konnte und daher auch keine Tg gemessen werden konnte. Mithilfe der Fox-Gleichung war es aber möglich „hypothetische Tgs“ von PHE für jede co-amorphe äquimolare Verbindung zu berechnen. Diese hypothetischen Tgs galten als Richtwerte um einerseits einschätzen zu können, wie stark die molekularen Wechselwirkungen zwischen jedem Arzneistoff und PHE sein würden und daraus folgend vorauszusagen, wie stabil jede der co-amorphen Verbindungen sein würde. Co-amorphe Verbindungen mit höher gemessenen Tgs und höher berechneten hypothetischen Tgs von PHE galten als stabile Systeme mit stärkeren molekularen Wechselwirkungen.

Verbindungen mit „niedrigen“ und „mittleren“ gemessenen Tgs zeigten in ihren Diffraktogrammen innerhalb weniger Tage ab Beginn ihrer Stabilitätsmessung Rekristallisationen einer der Substanzen an, während sich der amorphe Zustand der zweiten Substanz bis zum Ende der Studie nicht änderte. Einige Verbindungen waren trotz höher gemessenen Tgs und errechneten stärkeren Wechselwirkungen zwischen Wirkstoff und PHE ungenügend stabil, da beide Substanzen in kurzer Zeit wieder in ihren kristallinen Zustand übernahmen. Nur eine der acht co-amorphen binären Verbindungen blieb während der gesamten Durchführung (70 Tage) physikalisch stabil. Parallel zu den äquimolaren Proben wurden die amorphen Reinsubstanzen und restlichen co-amorphen Verbindungen bei Raumtemperatur („unkontrollierte Bedingungen“) gelagert. Die Idee dahinter war herauszufinden, ob co-amorphe Mischungen nicht-äquimolarer Mengen hinsichtlich ihrer Lagerung und Stabilität genauso nützlich oder sogar nützlicher wären als co-amorphe äquimolare Verbindungen.

Zusammenfassend zeigt die in dieser Arbeit beschriebene Studie, dass die Stabilität co-amorpher Mischungen bei äquimolaren Verhältnissen nicht allein von den gemessenen Tgs und / oder dem Ausmaß ihrer molekularen Wechselwirkungen abhängig ist. Neue Erkenntnisse legen nahe, dass die Wahl einer äquimolaren Mischung möglicherweise nicht immer die geeignetste ist, um co-amorphe Verbindungen herzustellen, die über einen längeren Zeitraum stabil bleiben sollen.

6.2. Ball-milling DATA

$$\text{Weight of } 50\% \text{ PHE} = \frac{(0{,}5 \ * \ mol\% \ PHE)}{[(0{,}5 \ * \ mol\% \ PHE) \ + \ (0{,}5 \ * \ mol\% \ drug)\}}$$

Low-Tg Drugs

(252,34 g/mol) CIM-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	337 mg	**25%**	663 mg	360
50%	604 mg	**50%**	396 mg	180
75%	820 mg	**75%**	180 mg	180
100%	1 g	**100%**	-	90

(418,57 g/mol) SIM-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	458 mg	**25%**	542 mg	not amorphous
50%	717 mg	**50%**	283 mg	270
75%	884 mg	**75%**	116 mg	360
100%	1 g	**100%**	-	540

Medium-Tg drugs

(381,37 g/mol) CEL-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	435 mg	25%	565 mg	450
50%	698 mg	50%	302 mg	360
75%	874 mg	75%	126 mg	360
100%	1 g	100%	-	360

(357,79 g/mol) IND-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	420 mg	25%	580 mg	180
50%	684 mg	50%	316 mg	90
75%	866 mg	75%	134 mg	90
100%	1 g	100%	-	90

(406,48 g/mol) CAR-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	450 mg	25%	550 mg	360
50%	711 mg	50%	289 mg	90
75%	880 mg	75%	120 mg	90
100%	1 g	100%	-	90

High-Tg drugs

(330,745 g/mol) FUR-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	400 mg	**25%**	600 mg	180
50%	667 mg	**50%**	333 mg	180
75%	857 mg	**75%**	143 mg	180
100%	1 g	**100%**	-	360

(238,16 g/mol) NIT-PHE (165,19 g/mol)

	Drug		AA	BM time (min)
25%	325 mg	**25%**	675 mg	23 h
50%	590 mg	**50%**	410 mg	180
75%	812 mg	**75%**	188 mg	180
100%	1 g	**100%**	-	180

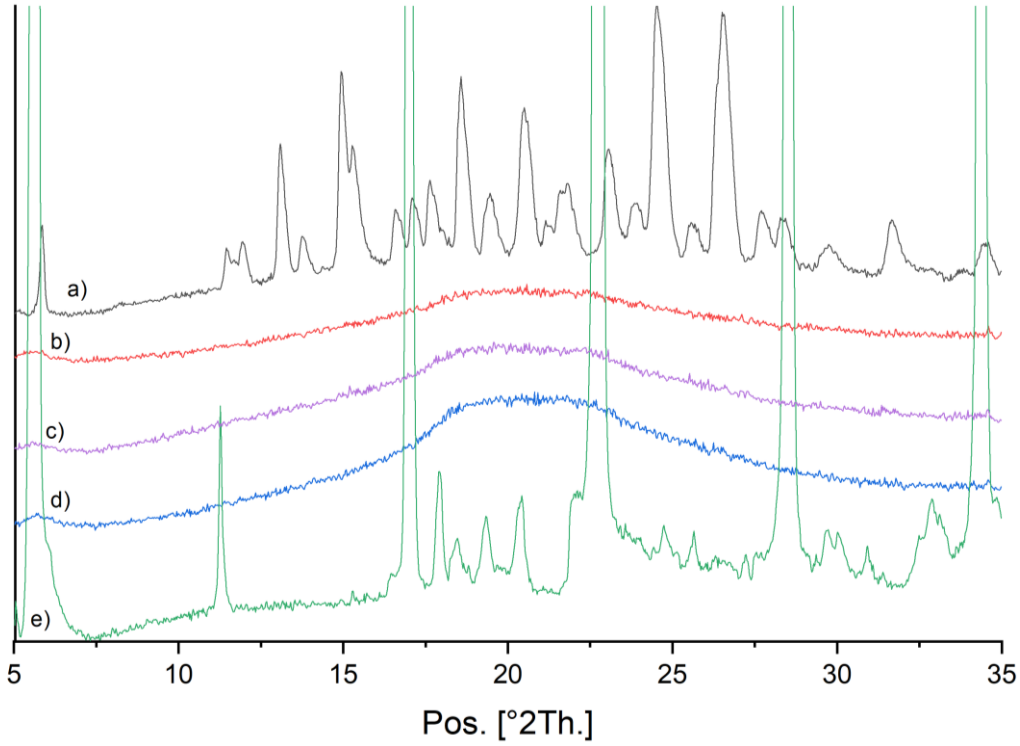
(295,29 g/mol) MEB-PHE (165,19g/mol)

	Drug		AA	BM time (min)
25%	373 mg	**25%**	627 mg	270
50%	641 mg	**50%**	359 mg	270
75%	843 mg	**75%**	157 mg	90
100%	1 g	**100%**	-	90

6.3. XRPD DATA

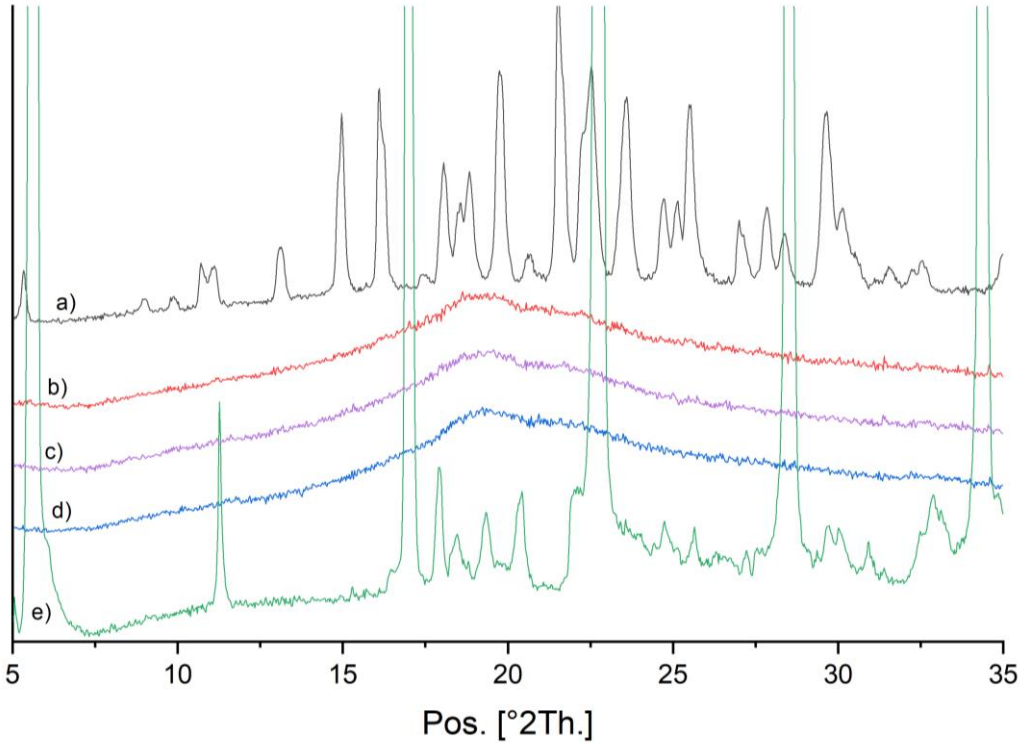
XRPD data of CAR-PHE

a) crystalline CAR; b) recrystallised at 40°C; c) recrystallised at RT; d) co-amorphous CAR-PHE; e) crystalline PHE



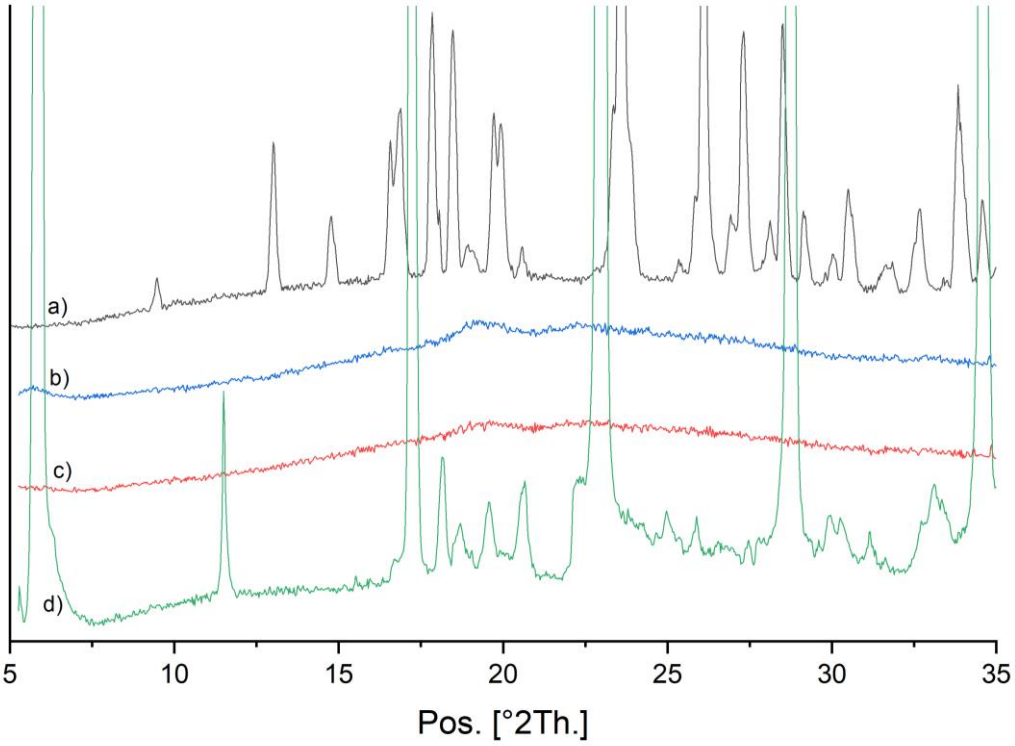
XRPD data of CEL-PHE

a) crystalline CEL; b) recrystallised at 40°C; c) recrystallised at RT; d) co-amorphous CEL-PHE; e) crystalline PHE



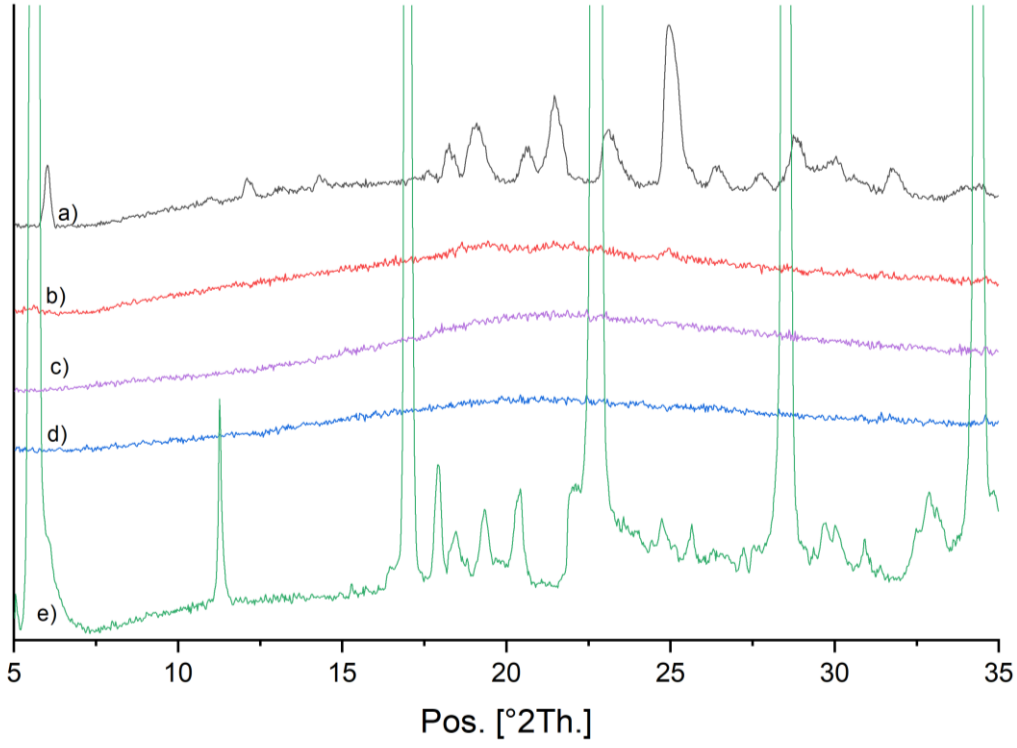
XRPD data of CIM-PHE

a) crystalline CIM; b) recrystallised at RT; c) co-amorphous CIM-PHE; d) crystalline PHE



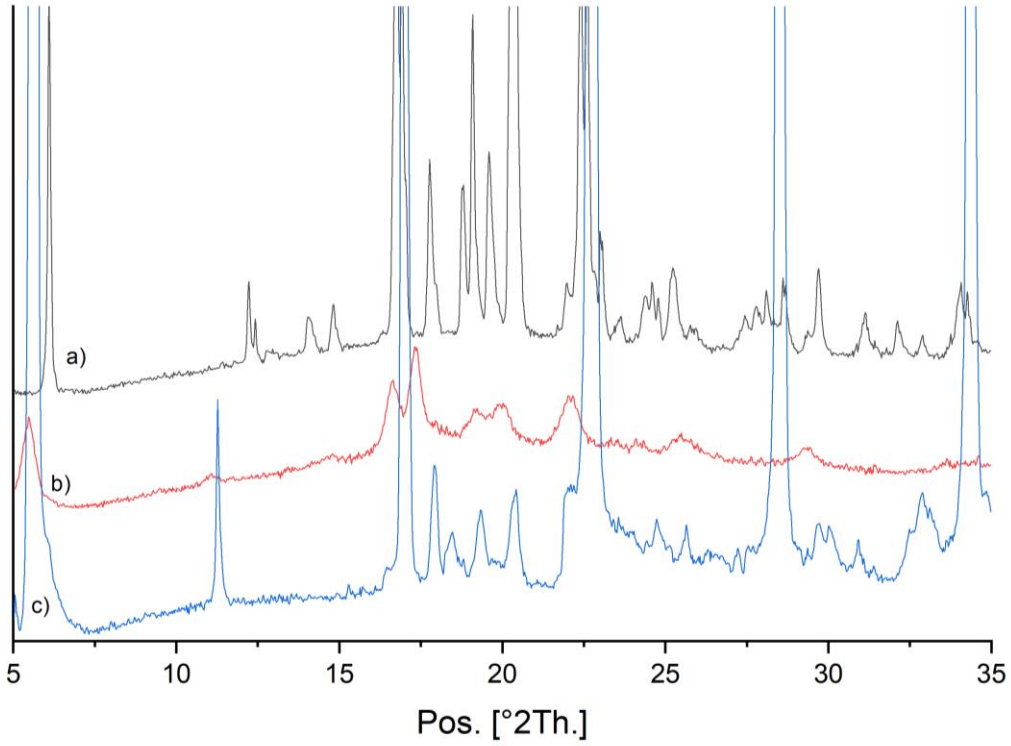
XRPD data of FUR-PHE

a) crystalline FUR; b) recrystallised at 80°C; c) stable at RT; d) co-amorphous FUR-PHE; e) crystalline PHE



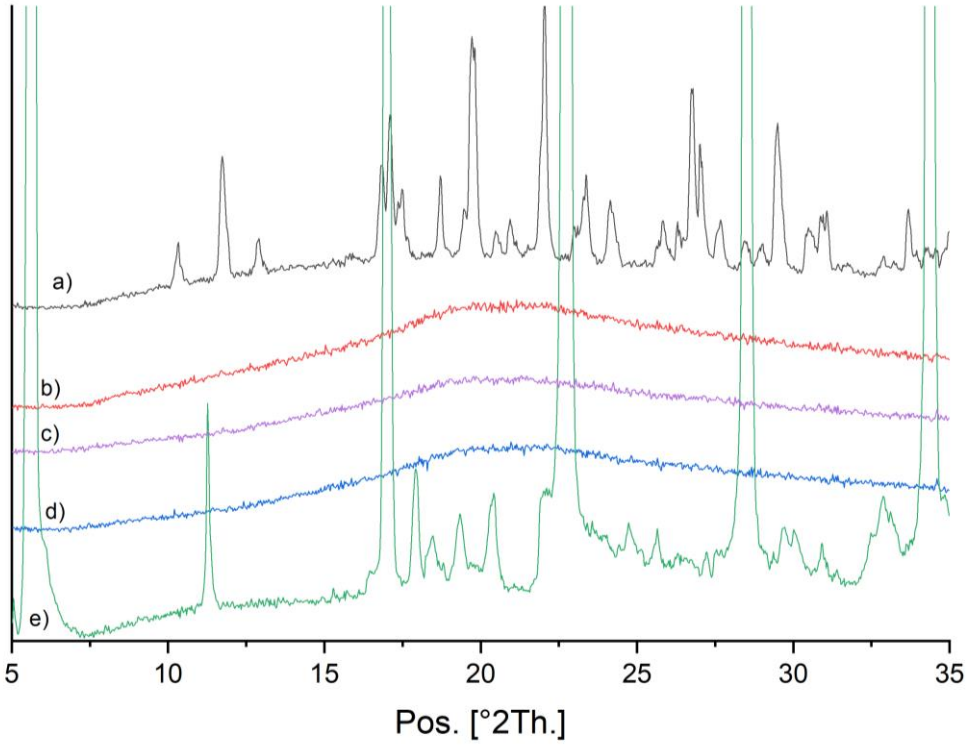
XRPD data of IBU-PHE

a) crystalline IBU; b) not co-amorphous IBU-PHE; c) crystalline PHE



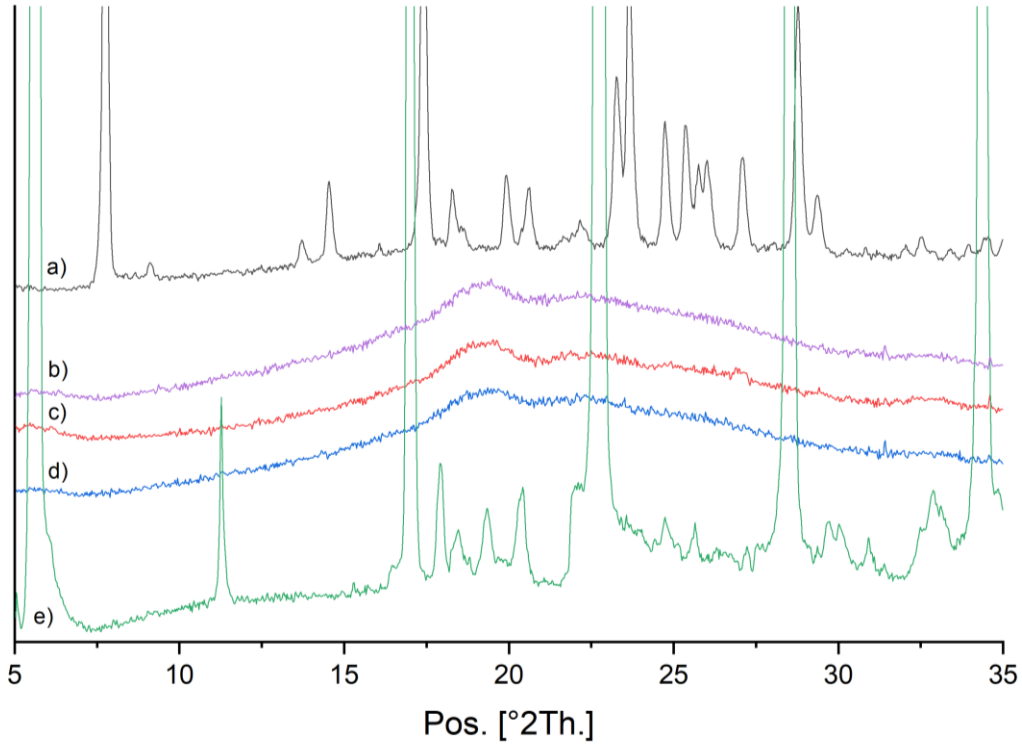
XRPD data of IND-PHE

a) crystalline IND; b) stable at 40°C; c) stable at RT; d) co-amorphous IND-PHE; e) crystalline PHE



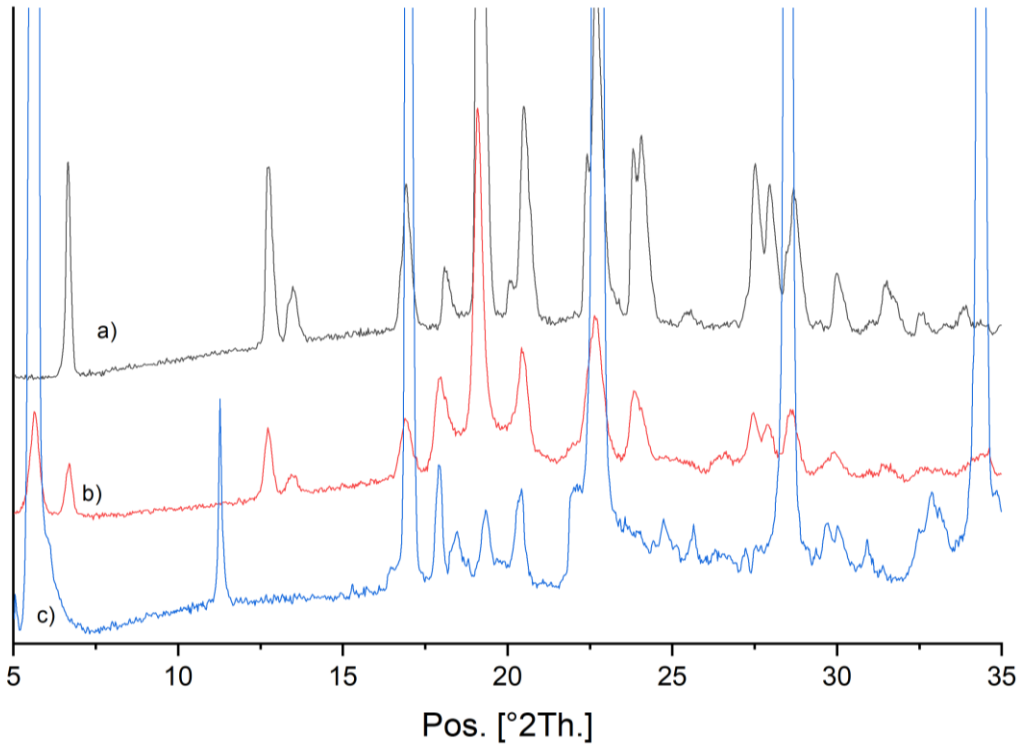
XRPD data of MEB-PHE

a) crystalline MEB; b) recrystallised at 80°C; c) stable at RT; d) co-amorphous MEB-PHE; e) crystalline PHE



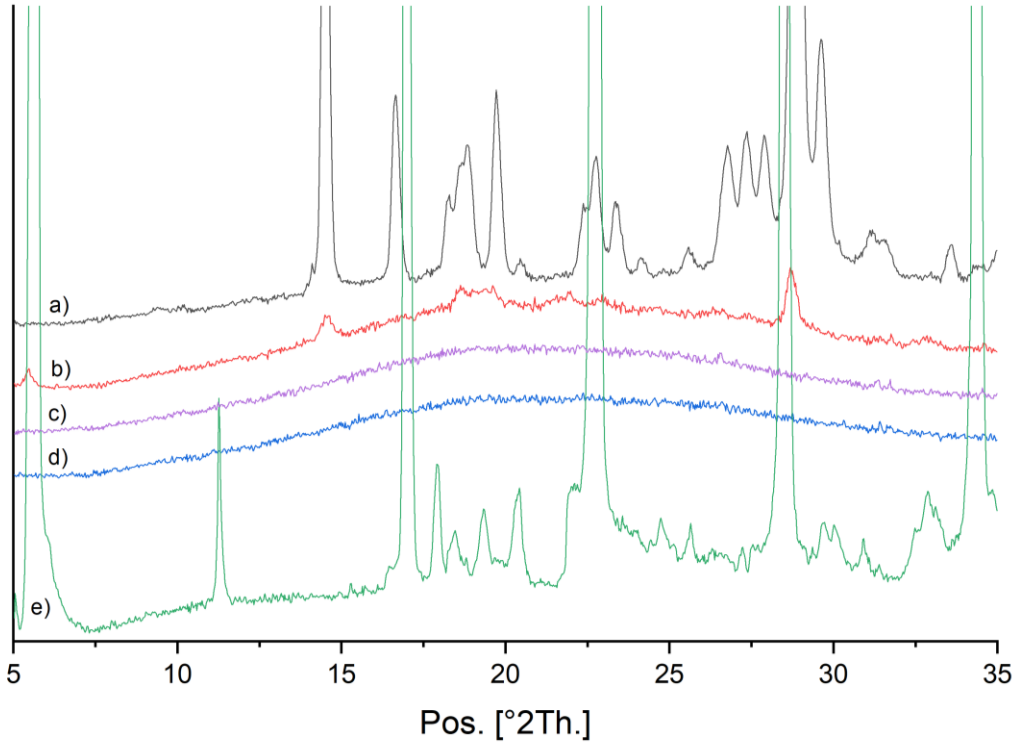
XRPD data of NAP-PHE

a) crystalline NAP; b) not co-amorphous NAP-PHE; c) crystalline PHE



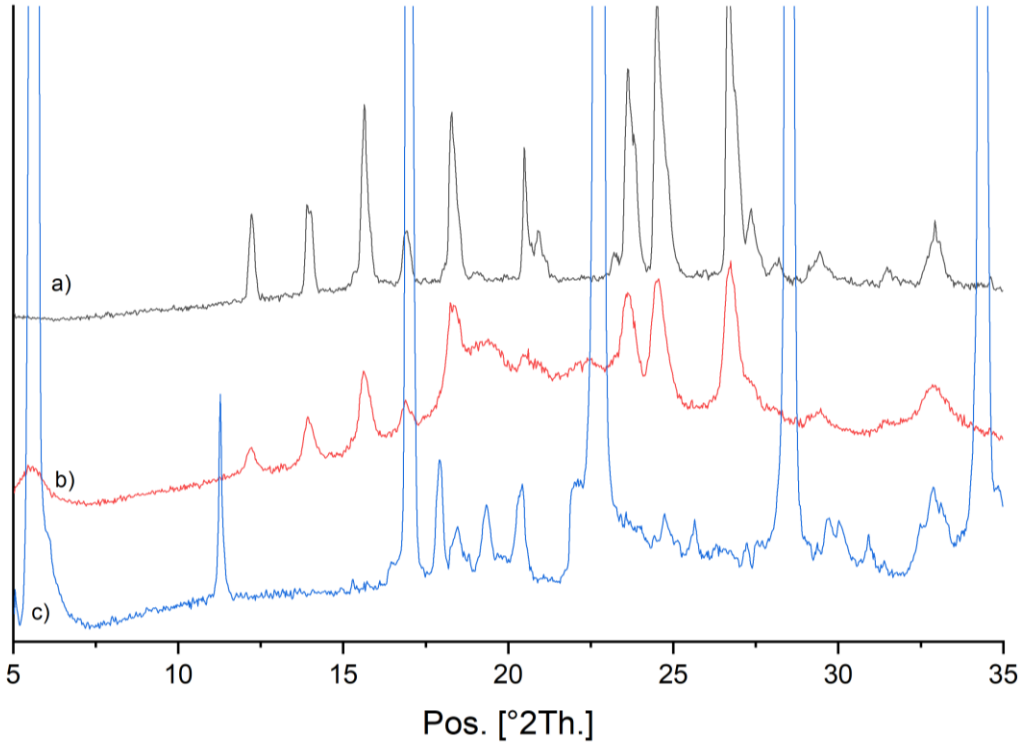
XRPD data of NIT-PHE

a) crystalline NIT; b) recrystallised at 80°C; c) stable at RT; d) co-amorphous NIT-PHE; e) crystalline PHE



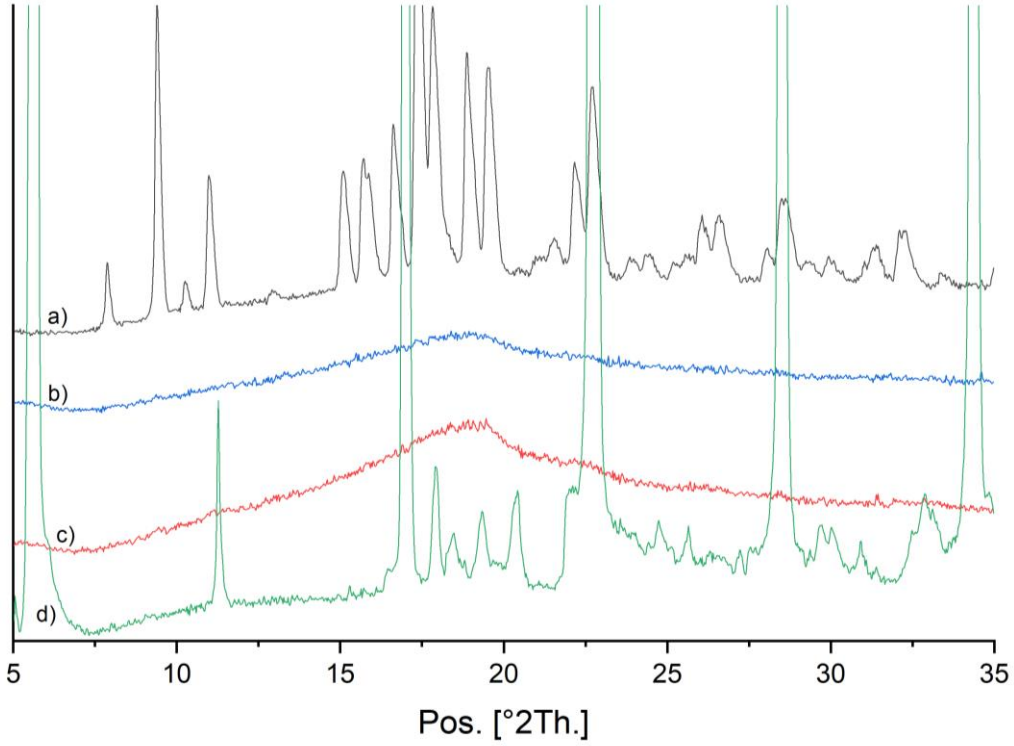
XRPD data of PAR-PHE

a) crystalline PAR; b) not co-amorphous PAR-PHE; c) crystalline PHE



XRPD data of SIM-PHE

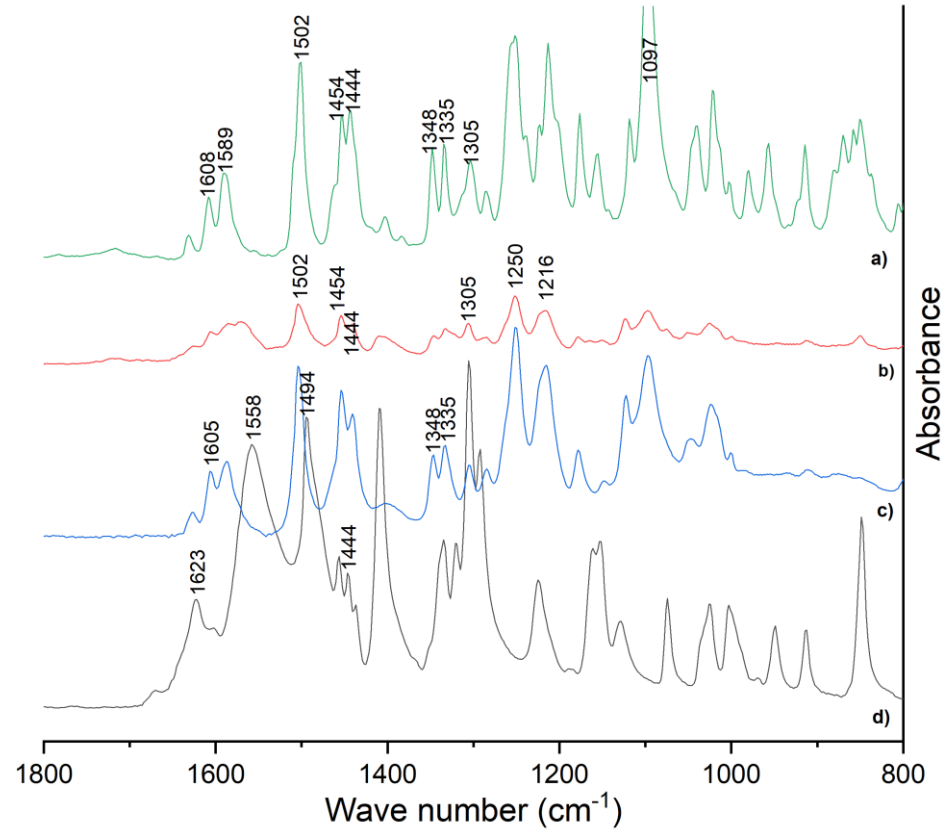
a) crystalline SIM; b) recrystallised at RT; c) co-amorphous SIM-PHE; d) crystalline PHE

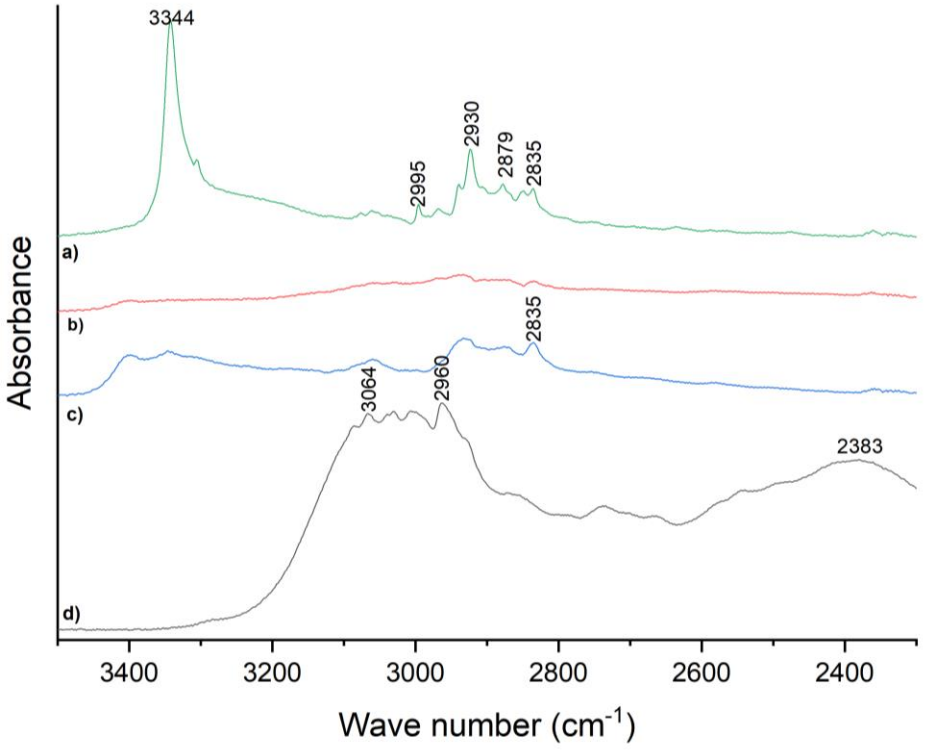


6.4. FTIR DATA

FTIR Data of CAR-PHE

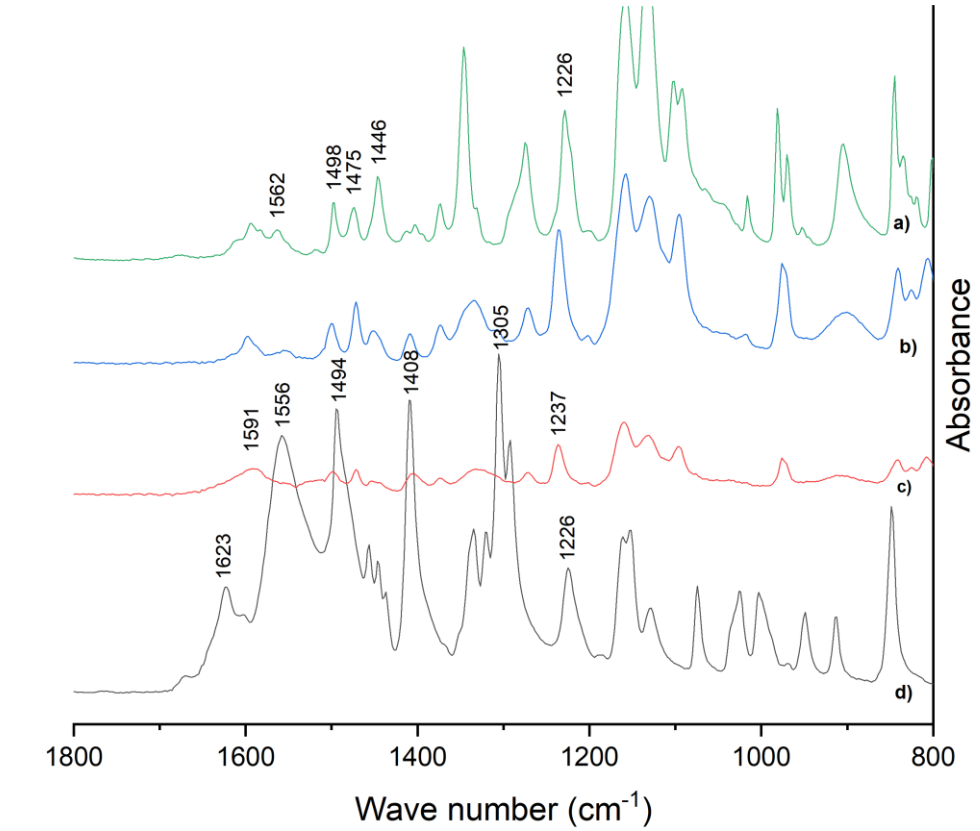
a) crystalline CAR; b) co-amorphous CAR-PHE; c) amorphous CAR; d) crystalline PHE

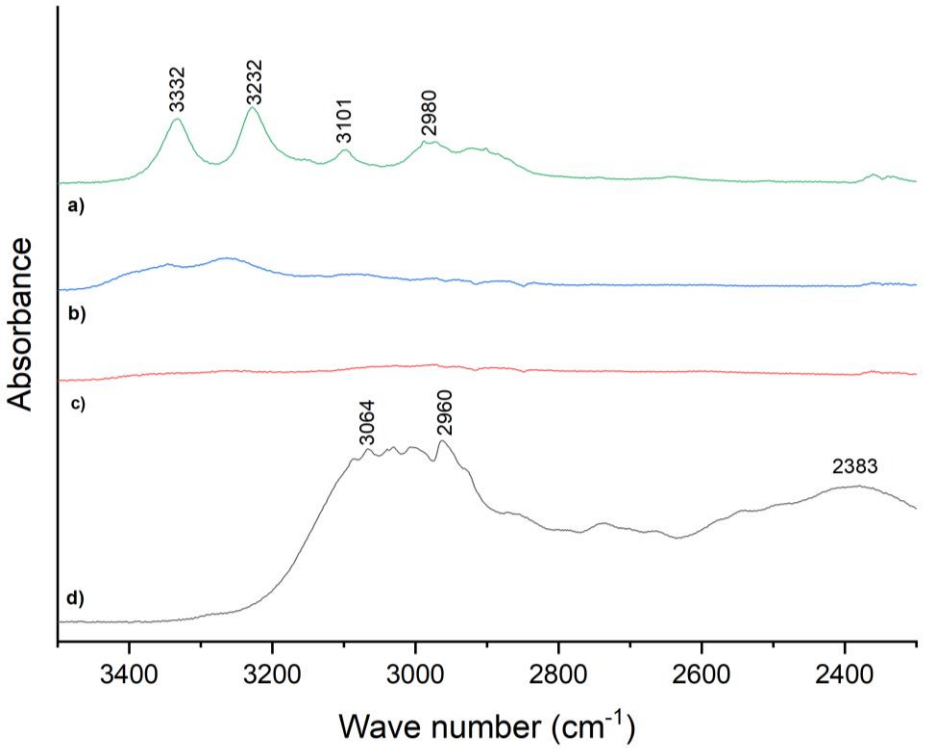




FTIR Data of CEL-PHE

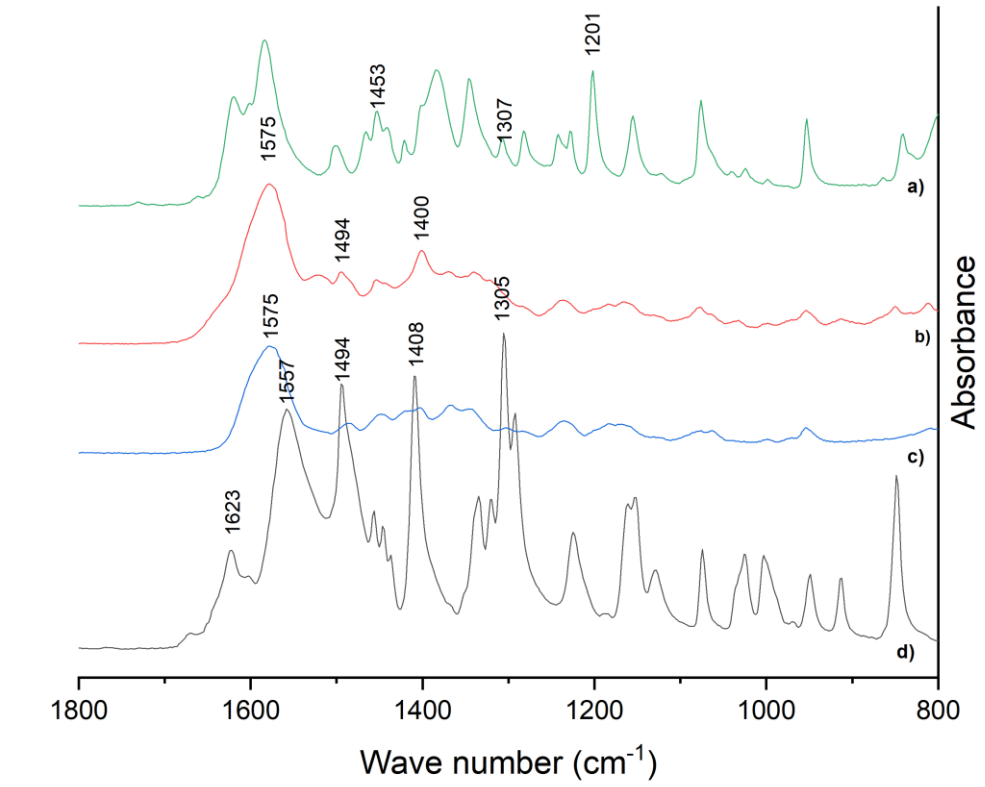
a) crystalline CEL; b) amorphous CEL; c) co-amorphous CEL-PHE; d) crystalline PHE

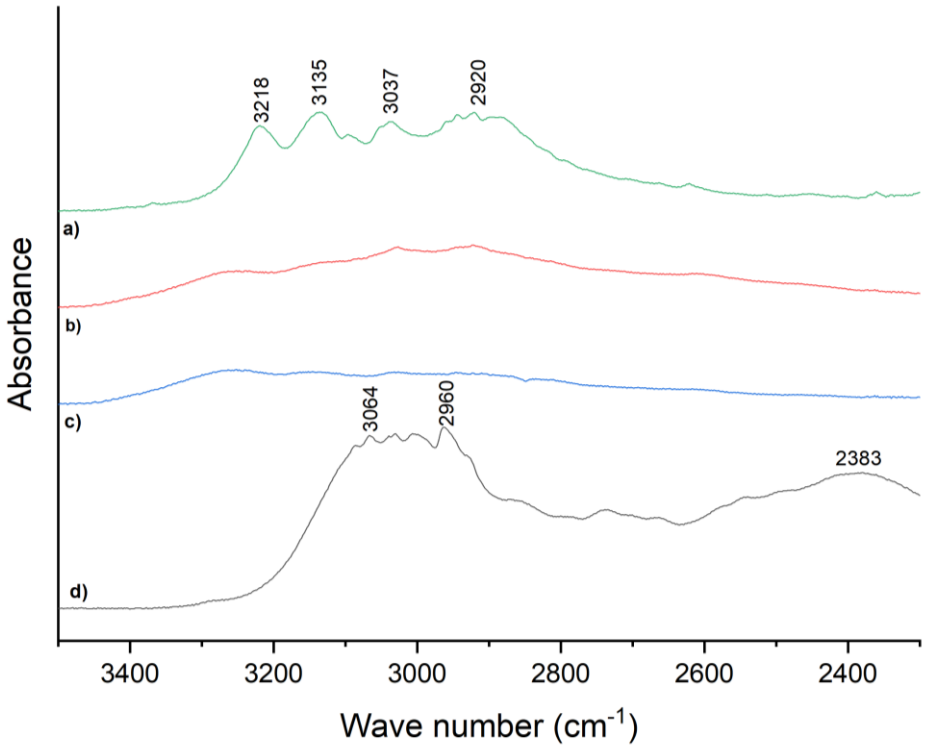




FTIR Data of CIM-PHE

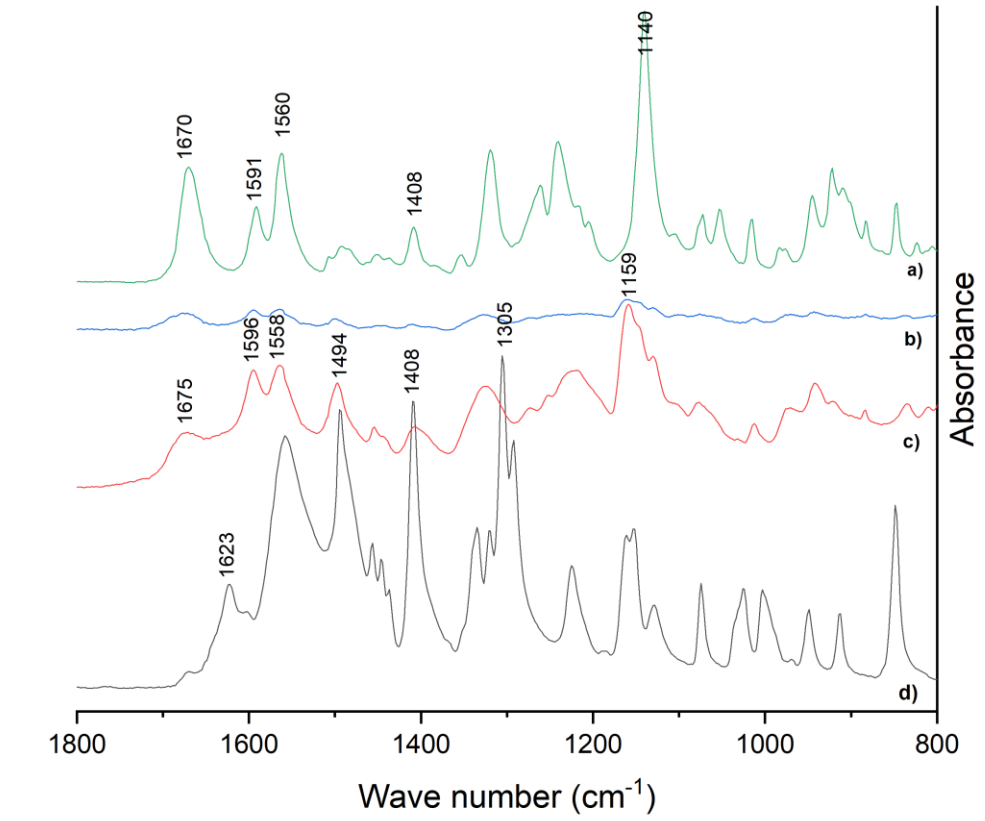
a) crystalline CIM; b) co-amorphous CIM-PHE; c) amorphous CIM; d) crystalline PHE

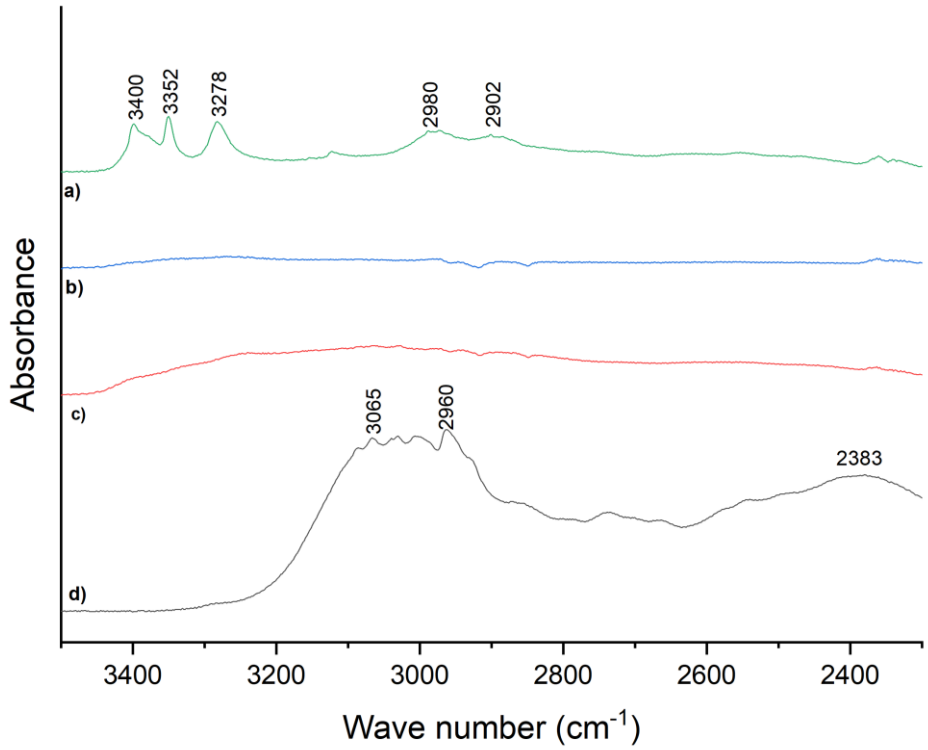




FTIR Data of FUR-PHE

a) crystalline FUR; b) amorphous FUR; c) co-amorphous FUR-PHE; d) crystalline PHE

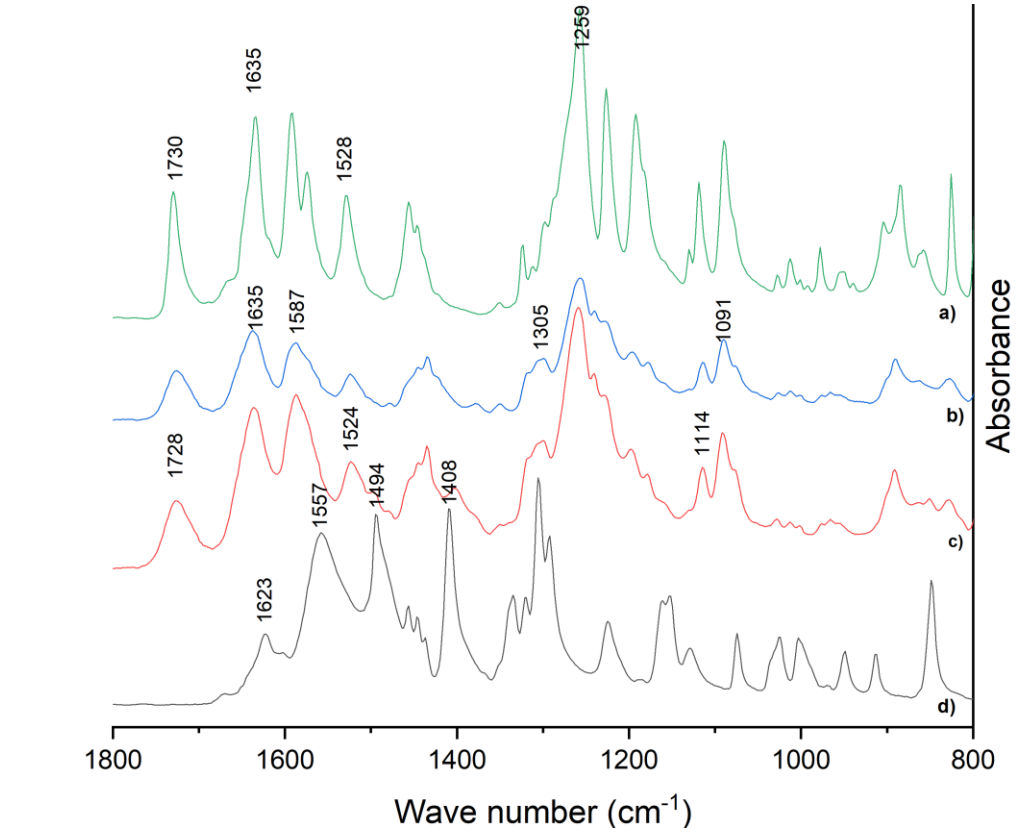


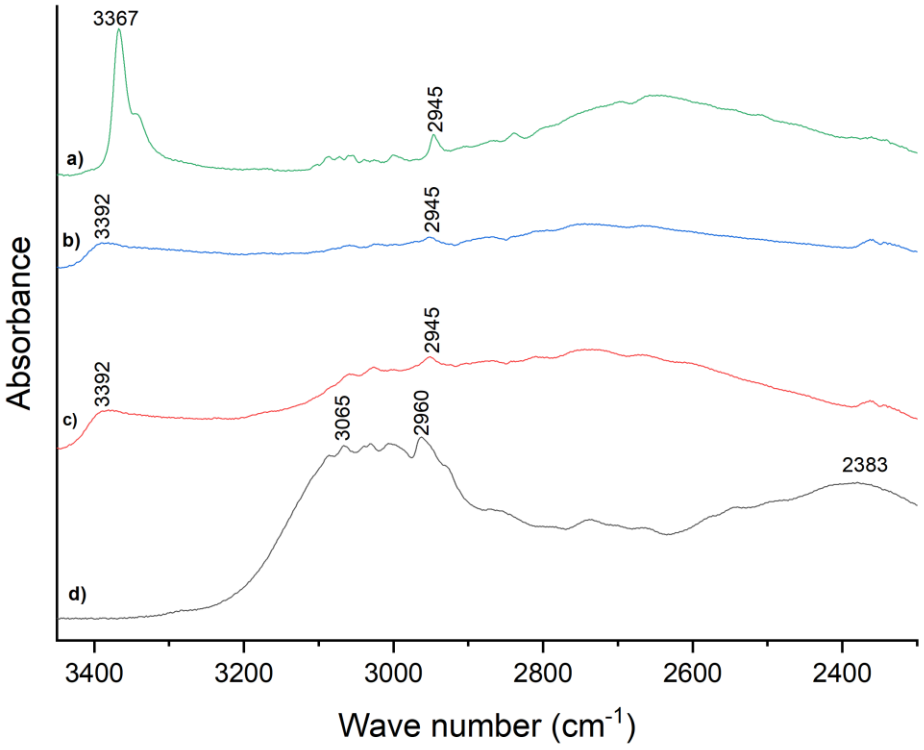


FTIR Data of IND-PHE on page 22

FTIR Data of MEB-PHE

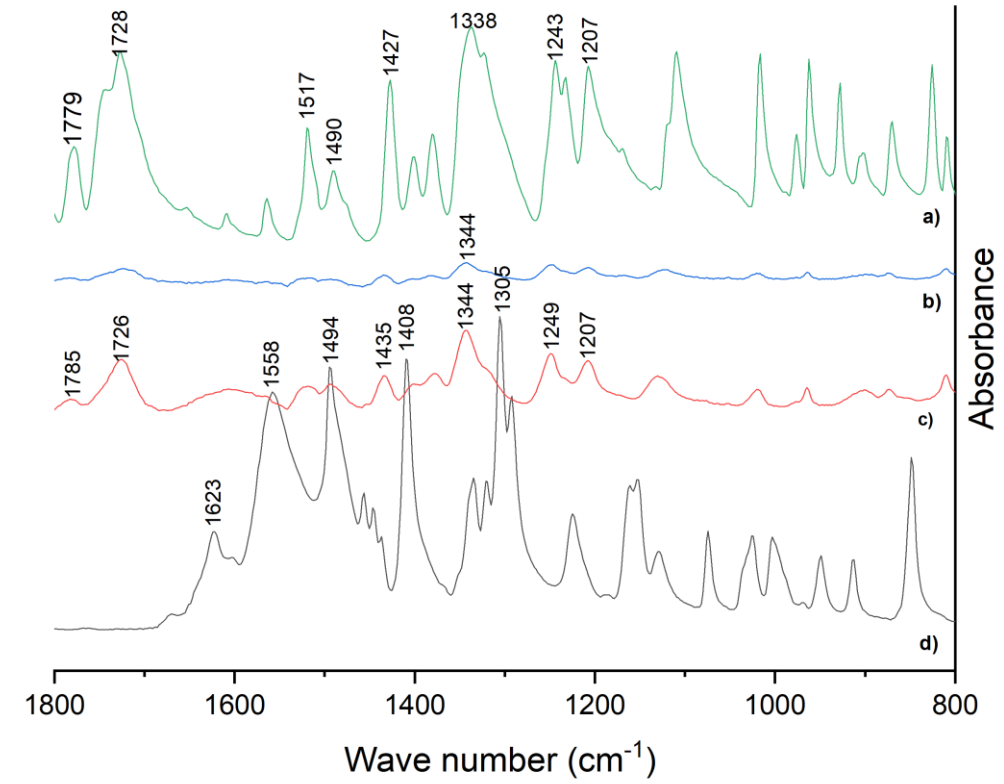
a) crystalline MEB; b) amorphous MEB; c) co-amorphous MEB-PHE; d) crystalline PHE

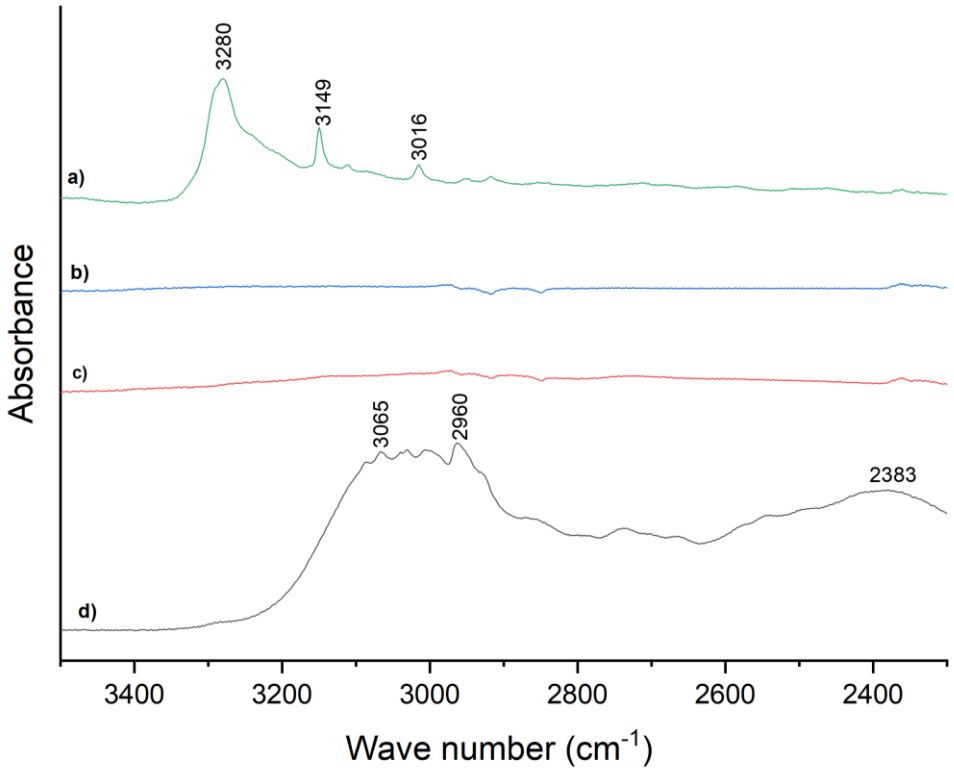




FTIR Data of NIT-PHE

a) crystalline NIT; b) amorphous NIT; c) co-amorphous NIT-PHE; d) crystalline PHE





FTIR Data of SIM-PHE

a) crystalline SIM; b) amorphous SIM; c) co-amorphous SIM-PHE; d) crystalline PHE