Division of Pharmaceutical Technology
Faculty of Pharmacy
University of Helsinki

# Expanding the Diversity of Solid-State Forms of Weak Bases by Applying Salt-Cocrystal Continuum Concept*:*

## *from Polymorphs and Solvates to Salts and Cocrystals*

Anna Shevchenko

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Pharmacy the University of Helsinki, for public examination in lecture room 1 at Info Center (Viikinkaari 11 A), Viikin campus,
on 22 November 2013, at 12 noon.

Helsinki 2013

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ISBN 978-952-10-9358-6 (paperback)
ISBN 978-952-10-9359-3 (PDF)

Helsinki University Printing House Helsinki 2013

Dedicated to the memory of my father Sergej Kazachkov

*О, сколько нам открытий чудных*
*Готовят просвещенья дух*
*И опыт, сын ошибок трудных,*
*И гений, парадоксов друг,*
*И случай, бог изобретатель.*

*А.С. Пушкин*

*Oh, how many wondrous revelations*
*Prepares for us the spirit of enlightenment,*
*Experience, the son of painful errors,*
*And genius, the paradoxes' friend,*
*And god of all inventions - simple chance.*

*A.S. Pushkin (translation from Russian)*

# Abstract

Poor physicochemical properties of pharmaceutical solids significantly restrain both pre-clinical development and clinical translation of investigational new drugs. Scientific studies that disclose more solid-state forms of a given drug compound are therefore very important for pharmaceutical industry. In regard to this, the need for effective solid-form screening approaches, especially designed for the early discovery phases, is widely recognized. The goal of the studies presented in this thesis is to elaborate an approach to expand the diversity of the solid-state forms of weak bases and enable efficient tailoring of their properties, especially solubility, hygroscopicity and physical stability.

The thesis introduces the results of a systematic research work on the discovering and characterizing new solid-state forms of weakly basic drug molecules. In particular, an effective and fast approach to initial evaluation of the polymorphism and solvatomorphism tendency and physical stability of drug candidates was developed. Using this approach, three crystalline forms, two anhydrous and one hemihydrate, of a hydrochloric salt were discovered. The most stable of the discovered forms, the hemihydrate, was selected for a future drug development. The introduced experimental procedures can be recommended also for evaluation of the polymorphism and solvatomorphism tendency of new chemical entities ("precandidates") even prior to the final selection of a drug candidate.

In order to enable the counterion and coformer selection for the salt and cocrystal formation, the exact knowledge of the crystal structure of the drugs and lead compounds is of particular importance. In our study, we have corrected the crystalline structure of the neat itraconazole, a potent antifungal drug, and the itraconazole-succinic acid cocrystal. The corrected data was used by us for preliminary selection of possible coformers promising in view of formation of cocrystals and salts with itraconazole.

In another study, the potential of the cocrystal formation of itraconazole with C2-C10 aliphatic dicarboxylic acids was investigated. Using a combination of two experimental screening techniques (solvent-drop grinding and slow evaporation), we have successfully synthesized the cocrystals of itraconazole with C2-C7 acids. These include anhydrous cocrystals (malonic, succinic, glutaric and pimelic acids), a cocrystal hydrate (adipic acid), and cocrystal solvates with acetone and tetrahydrofuran (oxalic acid). Most importantly, C7 was identified as the maximum carbon atom number of the aliphatic chain for the successful cocrystallization reaction between itraconazole and a dicarboxylic acid. This work demonstrates a wide diversity of itraconazole cocrystals with aliphatic dicarboxylic acids having a variety of the carbon chain lengths. This finding alone has a considerable conceptual and also practical value in the field of crystal engineering, putting an additional emphasis on the importance of the weak intermolecular interactions in the crystal structure cohesion.

A comparative evaluation of a new cocrystal of itraconazole with malonic acid and two new hydrochloric salts (dihydrochloride and trihydrochloride) of itraconazole has also been performed. The intrinsic dissolution rate, hygroscopicity, and thermodynamic stability were determined for the obtained solid-state forms and compared to itraconazole-succinic acid (2:1) cocrystal. The results show that, in general, the solid-state forms with higher intrinsic dissolution rate are less stable. However, the new cocrystal was found to

increase the dissolution rate of the parent drug by about 5-fold without compromising the hygroscopicity and the physical stability. This study demonstrates that, for dissolution rate enhancement of poorly water-soluble weak bases, cocrystallization is a more suitable approach than the formation of hydrochloric salts.

# Acknowledgements

The research summarized in this work has been carried out at the Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, and at the Research and Development Department of the Orion Corporation, in collaboration with the Laboratory of Organic Chemistry, Department of Chemistry, University of Jyväskylä.

I am indebted to my supervisor Prof. Jouko Yliruusi for his scientific support and wisdom guidance during the course of this work. I am sincerely grateful to my instructor and friend Dr. Inna Miroshnyk for providing scientific contribution to my study form the beginning to the end. I am grateful to Dr. Jorma Haarala, Dr. Nonappa, Prof. Erkki Kolehmainen and Prof. Manu Lahtinen for their invaluable contribution to this work. I thank also Dr. Lars-Olof Pietilä for his important help with molecular modeling. I wish to express my thanks to Dr. Bert van Veen and Prof. Juha Kiesvaara for arranging the practical matters and for productive discussions.

I thank also Dr. Jukka Salmia, Dr. David Din Belle, Mrs. Saara Tiittanen, Mr. Mikko Mäkelä, Dr. Luis Bimbo, Dr. Kaisa Syrjänen, Dr. Helder Santos, Dr. Sabbirudin Mirza, Mr. Kai Sinervo, Prof. Anne Juppo, Mrs. Krista Ojala, Mrs. Johanna Husman-Piirainen and Mrs. Kirsi Salomäki for their collaboration and support. I am also thankful to the managers of the Laboratory of Physics Mrs. Sanna Peltoniemi, Dr. Tero Närvänen, Dr. Paula Lehto and Mrs. Eerika Scarp and the manager of the Medicinal Chemistry Department Mrs. Leena Otsomaa at Orion for making my doctoral studies possible. My special gratitude goes to Prof. Veli-Pekka Tanninen, one of the founders of the Laboratory of Physics at Orion, who has succeeded in creating a fantastic inspiring working atmosphere. I also thank the reviewers of my thesis Prof. Niklas Sandler and Prof. Jyrkki Heinämäki for their valuable comments.

I thank my husband Andriy and son Kirill for their love and continuous support and help. I also thank my beloved mother Irma for her measureless love, understanding and support of all my initiatives. I own this work for the sweetest memories of my Best in the World father Sergej.

I thank all members of my extended family for sharing with me the adventure of repatriation to Finland. I also thank Dr. Marko Hämäläinen and Mr. Jukka Suomalainen who were my first friends and supporters here in Finland. I am grateful to all my Ukrainian friends, especially Olena Uskova and Andriy Pshenichniy, for providing a nice counterbalance to my work.

I acknowledge the financial support from Orion Corporation and TEKES during first two years of the research

Espoo, September 2013
Anna Shevchenko

# Contents

# List of original publications

This thesis is based on the following publications:

I Anna Shevchenko, David Din Belle, Saara Tiittanen, Arto Karjalainen, Arto Tolvanen, Veli Pekka Tanninen, Jorma Haarala, Mikko Mäkelä, Jouko Yliruusi, and Inna Miroshnyk. Coupling polymorphism/solvatomorphism and physical stability evaluation with early salt synthesis optimization of an investigational drug. *ACS*, Organic Process Research and Development, 2011, 15 (3), 666–672.

II Nonappa, Manu Lahtinen, Erkki Kolehmainen, Jorma Haarala, and Anna Shevchenko. Evidence of weak halogen bonding: New insights on itraconazole and its succinic acid cocrystal. *ACS*, Crystal Growth & Design, 2013, 13 (1), 346–351.

III Anna Shevchenko, Inna Miroshnyk, Lars-Olof Pietilä, Jorma Haarala, Jukka Salmia, Kai Sinervo, Sabbirudin Mirza, Erkki Kolehmainen, Nonappa and Jouko Yliruusi. Diversity in itraconazole cocrystals with aliphatic dicarboxylic acids of varying chain length. *ACS*, Crystal Growth & Design, 2013. Just published. DOI: 10.1021/cg401061t

IV Anna Shevchenko, Luis M. Bimbo, Inna Miroshnyk, Jorma Haarala, Kristýna Jelínková, Kaisa Syrjänen, Bert van Veen, Juha Kiesvaara, Hélder A. Santos, Jouko Yliruusi. A new cocrystal and salts of itraconazole: comparison of solid-state properties, stability and dissolution behavior. International Journal of Pharmaceutics, 2012, 436 (1–2), 403–409.

The publications are referred to in the text by their roman numerals.

# Abbreviations

| | |
|---|---|
| ACD | Advanced Chemistry Development |
| ACE | Acetone |
| ACS | American Chemical Society |
| ADI | Adipic acid |
| API | Active pharmaceutical ingredient |
| AUC | Area under curve |
| BCS | Biopharmaceutics Classification System |
| CLF | Chloroform |
| Cmax | Maximum concentartion of the drug in blood |
| COSY | correlation spectroscopy (2D NMR) |
| CPMAS | Cross polarization magic angle spinning |
| CSD | Cambridge structure database |
| DMSO | Dimethyl sulfoxide |
| DMSO-d6 | Deuterated dimethyl sulfoxide |
| DSC | Differential scanning calorimetry |
| etc. | et cetera (and so on) |
| e.g. | exempli gratia (“for example”) |
| et al. | et alii (and others) |
| i.e. | id est (in other words) |
| GLU | glutaric acid |
| GVS | Gravimetruc vapor sorption |
| $H_2O$ | Water |
| HCl | Hydrochloric acid |
| HPLC | High performance liquid chromatography |
| HMBC | Heteronuclear multiple-bond correlation spectroscopy |
| HSQC | Heteronuclear single quantum correlation |
| HT | High Throughput (screening) |
| ICDD | International Centre for Diffraction Data |
| IDR | Intrinsic dissolution rate |
| IR | Infrared spectroscopy |
| ITZ | Itraconazole |
| IUPAC | International union of pure and applied chemistry |
| LI | Lead identification |
| LO | Lead optimisation |
| MAL | Malonic acid |
| MS | Mass spectroscopy |
| NIR | Near infrared spectroscopy |
| NMR | Nuclear magnetic resonance spectroscopy |
| OXA | Oxalic acid |
| PhD | Philosophiae doctor |
| PIM | Pimelic acid |
| Raman | Raman spectroscopy |

| | |
|---|---|
| PDF | Powder Diffraction File |
| RH | Relative humidity |
| Ph. Eur. | European Pharmacopeia |
| PXRD | Powder x-ray diffractometry |
| ROESY | Rotating frame nuclear Overhauser effect spectroscopy |
| SSCI | Solid State Chemical Information laboratory |
| SSNMR | Solid state nuclear magnetic resonance |
| SUC | succinic acid |
| TCD | Thermo-conductivity detector |
| Teraherz | Teraherz spectroscopy |
| TGA | Thermogravimetry |
| TGA-MS | Synchronized TGA with head space mass spectroscopy |
| THF | Tetrahydrofuran |
| TMS | Tetramethylsilane |
| TPD | Temperature programmable desorption |
| TPPM | Two-pulse phase-modulated |
| UV-VIS | Ultraviolet visible spectrophotometry |

# 1 Introduction

The pharmaceutical science recognized the importance of solid-state properties of drugs starting from 1969 (Haleblian and McCrone, 1969). The turning point in this respect was a dramatic event of temporary removing the Norvir drug from the market in 1998 (Bauer *et al*. 2001). The dosage forms, soft-gel capsules, contained a solution of ritonavir, protease inhibitor, that started to crystallize upon shelf-life into more stable undiscovered polymorphic form. This affected the solubility and bioavailability of the drug and consequently the clinical effect of the product (Bauer *et al*. 2001, Gardner *et al.* 2004). Since then the control and study of solid-state properties of API's have become obligatory and been guided and supervised by Drug Regulatory Authorities. It is well known that various solid-state forms exhibit different physicochemical properties, such as apparent solubility, dissolution rate, chemical stability, physical stability, melting point, hygroscopicity, color, filterability, density, flow behavior, and many other properties (Byrn *et al.* 1995, 2010, Kato and Kohketsu 1981; Hancock *et al.* 2002; Brittain 2006, 2009; Gavezzotti 2007; Henck and Byrn 2007; Bernstein 2011) The role of the solid-state properties of drugs is of particular importance for low soluble drug candidates (Huang and Tong 2004; Balbach and Korn 2004), for which a small difference in solubility can significantly affect the bioavailablity of the drug (Higuchi *et al.* 1963; Aguiar and Zelmer 1969; Stahly 2007). Since high throughput screening and combinatorial synthesis methodology has been incorporated into the drug discovery process, number of drug candidates having low aqueous solubility grows constantly (Lipinski 2000). Thus there is a growing need to optimize and control solid state properties as early as possible.

Ideally, the in-vivo efficiency, pharmacokinetics, and toxicology studies of low soluble drug candidates should be performed with the drug candidates that exhibit optimal solid-state properties (Huang and Tong 2004; Steele 2009). This means that the most appropriate time for finding the most stable and soluble solid form is during the Lead Identification (LI) and Lead Optimization (LO) phases of the drug discovery process (Huang and Tong 2004; Balbach and Korn 2004; Steele 2009;). At the later stages, the developability assessment criteria for new drug candidates are commonly applied to identify the potential challenges and even project "stoppers" of the drug development (Huang and Tong 2004; Balbach and Korn 2004; Steele 2009). These criteria are typically set to solubility and dissolution, hygroscopicity, stability, and synthesis process scalability of the chosen solid-state form. Estimation of scalability of the synthesis process and physical stability includes evaluation of tendency of a new chemical entity to crystallize in different crystal forms, investigation of thermodynamic relationship between polymorphs as well as relative stability of possible polymorphic forms at ambient conditions (Llinas and Goodman 2008).

On the other hand, it is of importance to optimize the drug discovery and development process in order to fasten the market entry of the new drugs (Hariharan *et al.* 2003). According to (Henck and Byrn 2007; Byrn *et al.* 2010;) one of the key elements of the strategy to reduce the time required for preclinical development is to link solid-form discovery with formulation, formulation design and manufacturing. According to the authors of (Henck and Byrn 2007; Byrn *et al.* 2010), the most efficient way to speed up

new drug development is to accelerate the process of the ‘proof-of-concept’ (POC). In their opinion, such acceleration can be achieved by performing the Best Solid Form – Screenings as soon as the candidate has been selected. Knowledge of the polymorphism and solvatomorphism tendency and solid state properties of the new candidate is of great importance for screening facilitation.

To summarize, there is a need to expand the diversity of the solid-state forms to enable tailoring of their properties, especially solubility, in order to facilitate the selection of the most appropriate solid form already in the preclinical stage of the drug discovery and development process. The objectives of this study were (1) to obtain new developable forms of weakly basic drug molecules, (2) to assess the feasibility of the modeling tools for selecting coformers and counter-ions, (3) to develop effective screening approaches that can be used in industry for salts and cocrystals, (4) to evaluate the solubility, dissolution and stability of the new forms in order to select the solid-state forms with appropriate properties.

# 2 Theory and literature review

## 2.1 Classification and definitions

### 2.1.1 Biopharmaceutics Classification System (BCS)

Drug substances are traditionally classified with respect to their bioavailability under four classes according to the Biopharmaceutics Classification System (BCS) ( Amidon *et al.* 1995):

- Class I – high solubility and permeability;
- Class II – low solubility and high permeability;
- Class III – high solubility and low permeability
- Class IV – low solubility and low permeability

The strategies for improving solubility and maximizing the dissolution rate of low soluble drug molecules include, for example, particle size reduction to increase the surface area for dissolution (Rasenack 2002; Dai *et al. 2007*; Barrett,Angela 2008) solubilization of the drug in co-solvents (Kovács *et al.*. 2009) and micellar solutions (Yi *et al.* 2007), complexation with cyclodextrins (Bettinetti *et al.* 1989; deChasteigner *et al.* 1996; Alsarra *et al.* 2010), formulation of amorphous form dissolved in a polymer matrix (Verreck *et al.* 2003; Six *et al.* 2003; Engers *et al.* 2010) or incorporated into porous particles (Mellaerts *et al.* 2007; Kinnari *et al.* 2011), the use of lipid systems for the drug delivery (Yi *et al.* 2007), and formulation of eutectic mixture of a low soluble drug and well soluble excipient (Liu *et al.* 2006).

The best way to improve the solubility often depends on the physical and chemical nature of the drug. All the methods have their own strong and weak aspects as well as limitations and must be considered case-by-case, taking into account the scientific, technological and economical factors (Grant and Higuchi 1990; Steele 2009).

In this PhD thesis the focus is on the molecules of BSC Class II drug substances intending to show the ways of enhancing the dissolution and solubility of drug substances by tailoring the appropriate solid-state form.

### 2.1.2 Dissociation of weak electrolytes in aqueous solutions and pH solubility profiles of acidic and basic drug substances

Approximately two-thirds of all existing drug substances are weak electrolytes that, in aqueous solution, exist as ions (Hancock *et al.* 2002). Such ions are formed as a result of

release or acceptance of protons for acids and bases, respectively. For a monoprotic acid, the following dissociation reaction can be written:

$$HA + H_2O = H_3O^+ + A^- .$$

The degree to which the acid *HA* dissociates in a dilute solution is determined by the following equilibrium constant $K_a$:

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} .$$

Here, the brackets stand for molarity (concentration in moles/liter). In the dissociation reaction, water acts as a base, accepting a proton from the acid. The larger is the dissociation constant, the stronger is the acid and, simultaneously, the higher is its tendency to give up a proton. For convenience, the strength of an acid is indicated by the value of $pK_a$ defined as

$$pK_a = -\log K_a .$$

Analogously, for a monoprotic base one can write

$$BH^+ + HO^- = H_2O + B ,$$

and

$$K_a = \frac{[H^+][B]}{[BH]} .$$

Here, water acts as an acid accepting a proton from the base.

To be able to use the same quantities, $K_a$ and $pK_a$, for both acids and bases, the protonated base is regarded as the corresponding acid of the free base. Owing to this, acids and bases are classified by using their pKa as a strength-indicating parameter, see Table 1. (Stahl 2002).

**Table 1.** *Classification of Acids and Bases According to Strength. (Modified from (Stahl 2002))*

| | pKa | |
|---|---|---|
| Attribute | Acids | Bases |
| Very strong | <0 | 14 |
| Strong | 0-4.5 | 9.5-14 |
| Weak | 4.5-9.5 | 4.5-9.5 |
| Very weak | 9.5-14 | 0-4.5 |
| Extremely weak | >14 | <0 |

The aqueous solubility of the weak electrolyte will be maximal when all molecules are ionized and it will be minimal if the molecules are not ionized. Moreover, for a basic drug the maximum solubility will be reached at pH $\leq (pKa)_{base}$-2 and for an acidic drug at pH $\geq (pKa)_{acid}$+2 (Figure 1). The pH value in the human gastrointestinal tract is 1-3 in stomach, 5-7 in duodenum and jejunum, 7-8 in ileum and rectum. Consequently, for bases having pKa < 6 a low solubility is expected within the whole biological pH range.

This study has been focused on the solid-state form modification approaches for weak and very weak bases.

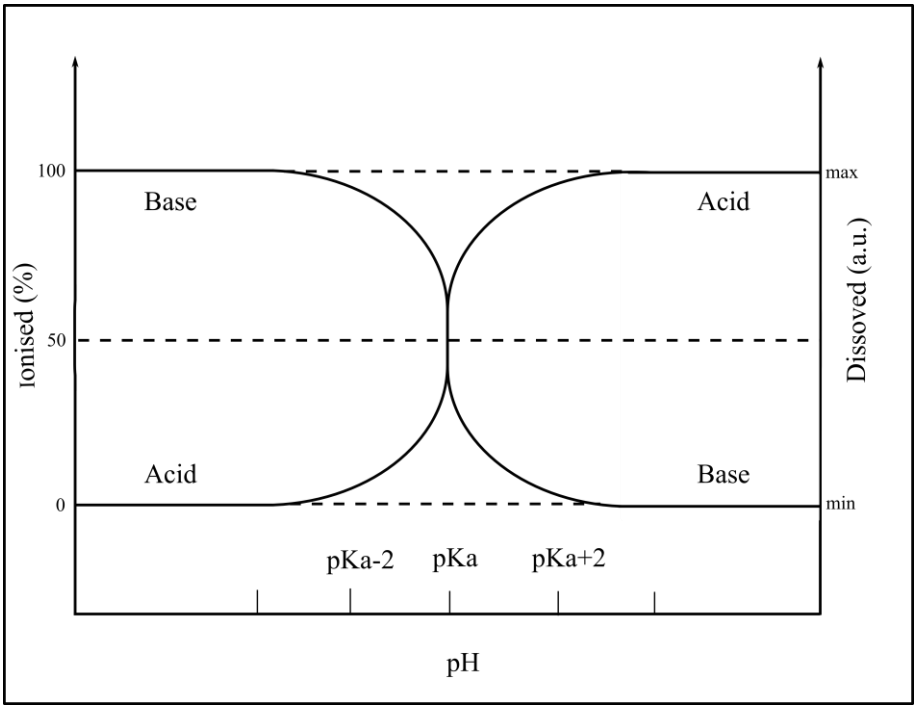



*Figure 1 Ionization % and solubility of acidic and basic drug molecule. Adopted from (Florence 2006).*

### 2.1.3 Solid-state forms

A scheme for classification of solid-state forms of drug molecules is presented in Figure 2. The crystals of a given substance may vary in size and have different shapes. In other words, they may have the same internal, but different external structures (Zhang and Zhou 2009). The external structure, however, does not determine the solid-state form.

If the internal structure of a substance has a long-range order and can be described by the structure's unit cell and certain elements of symmetry, the substance is crystalline. It is then possible to construct the crystal by repeating the unit cell in all three directions of space. In contrast, amorphous solids have only a short-range order and cannot be modeled by the above procedure.

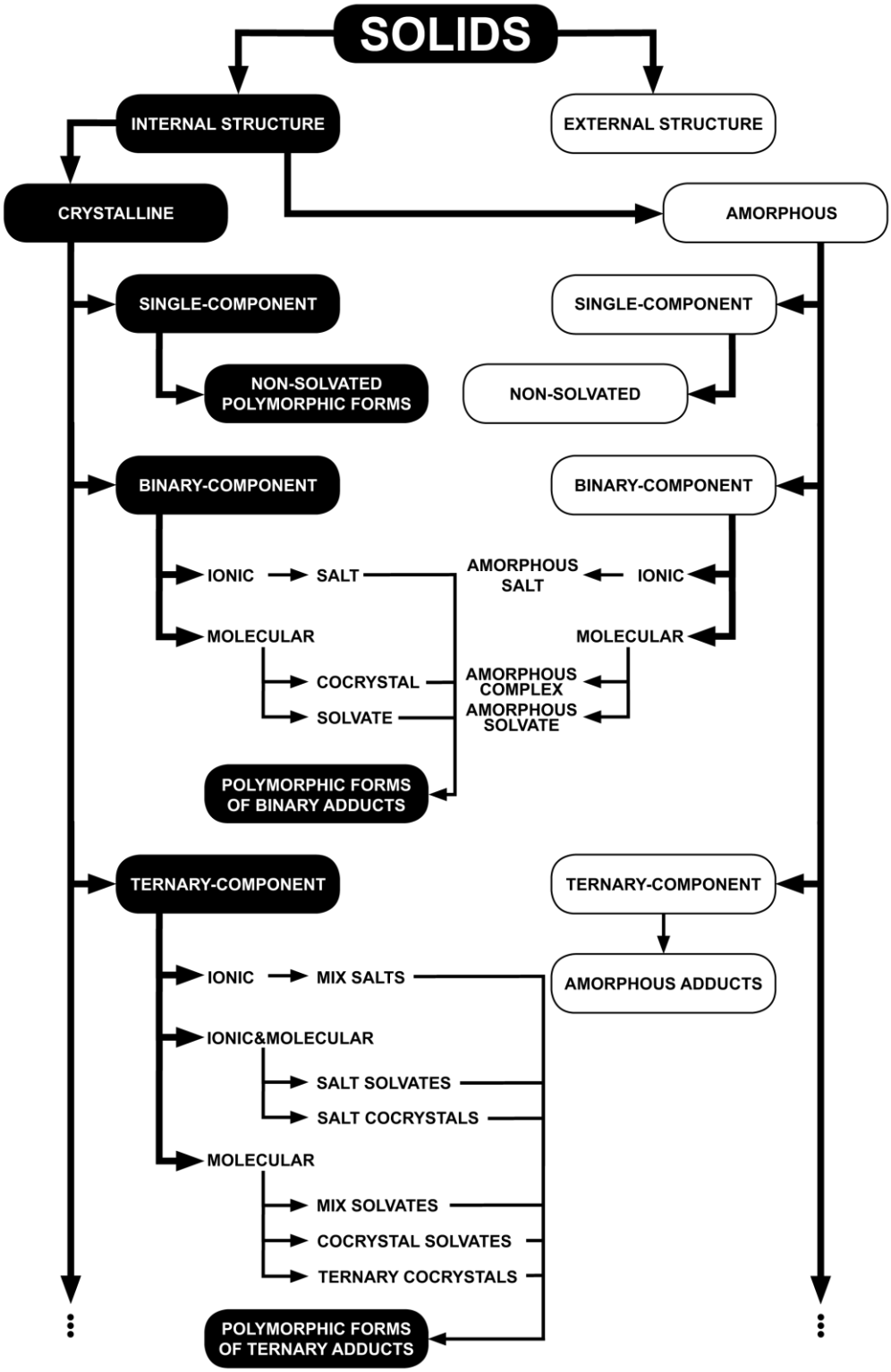



*Figure 2* *Classification of solid-state forms. Modified from (Haleblian 1969; Zhang and Zhou 2009; Zavorotko et al. 2012).*

Both amorphous and crystalline solids may be composed of a single component or a number of different components, chemical species. Crystalline solids composed of different species are called multi-component complexes or adducts (Zavorotko et al. 2012). Thermodynamically, any adduct is always a homogeneous single phase that should not be confused with an inhomogeneous physical, eutectic or eutectoid mixture of several components. The eutectic mixture has a peculiar external structure and is characterized by a melting point that is always lower than the melting points of the neat components.

Two main classes of adducts, ionic and molecular, can be defined on the basis of the chemical bonding between the molecules. Ionic compounds are salts, while molecular compounds are solvates or cocrystals. If one of the components in a molecular compound is liquid at normal conditions, the compound is a solvate. In case the components are solids at normal conditions, the compound is a cocrystal (Aakeröy and Salmon 2005; Bond 2007). The bonds between nonionic molecules in the crystal can be the H-bonds, π-π bonds between aromatic rings or, weaker but not less important, halogen and Van-der-Waals bonds (Desiraju 2009).

When the number of components in the compound is larger than 2, there is an option for the compound to form mixed types of adducts. In this case, the cocrystal salts and cocrystal solvates, in addition to ternary salts and cocrystals, can be formed (Zavorotko *et al.* 2012). The diversity of possible combinations will grow with the number of components in the crystal. It should also be emphasized, that adducts may contain more than one drug molecules, as well as stereo- and isomers (Childs *et al.* 2004; Zavorotko et al. 2012).

The ability of the crystal's components to arrange themselves in different ways in the crystal lattice is called polymorphism. For pure chemical elements this ability is known as allotropism. The polymorphism is exhibited only in solid materials. Any difference between polymorphs disappears when the relatively weak bonds in the crystal lattice are destroyed by dissolving, melting or evaporation. Both single and multi-component crystals may exhibit polymorphism.

A polymorphism due to possible variations in the crystal packing is called the packing polymorphism. Polymorphism caused by the existence of different conformers of the same molecule is called conformational polymorphism (Rodriguez-Spong 2004).

### 2.1.4 Salt–cocrystal continuum

Salts belong to one of the most widely known and explored class of solid forms. Their physical and chemical properties are well described in many handbooks and review articles (Stahl 2002, Bond *et al.* 2012). Approximately half of the oral drug forms contain the salts of active ingredients. These pharmaceutical salts are typically more rapidly and better soluble in stomach and intestinal juices than non-ionic species, which makes them advantageous components in the solid dosage forms. When the new drug candidate is a weak base and there is an indication of its low solubility in water, the preparation of a salt of this drug is automatically considered as the first choice for enhancement of its aqueous solubility. Moreover, purification of drug molecules during their synthesis is usually

performed by the salt preparation. Consequently the preparation and selection of the most appropriate salt is an important step in the drug development process (Stahl 2002, Bond *et al.* 2012).

Being discovered already more than 40 years ago, organic cocrystals still remain relatively unknown molecular compounds for pharmaceutical industry (Almarsson and Zavorotko 2004; Stahly 2007; Childs and Zavorotko 2009). There are still no drug forms on a market officially denoted as cocrystals, however there are new patents and patent applications (Almarsson *et al.* 2012), for instance, WO2011067571 (2011) of Astra Zeneca that claims cocrystals of the investigational drug candidate {1S- [1α, 2α, 3β (l,S* ,2R*), 5β]} -3 -(7 -{[ 2 -( 3,4-difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3H-l,2,3-triazolo[4,5-d]pyrimidin -3-yl)-5-(2-hydroxyethoxy)cyclopentane-l,2-diol, with a number of carboxylic acids. In spite of this, the opportunities the cocrystals can offer, such as the possibility to obtain additional multicomponent solid forms of weakly and non-ionisable molecules with improved pharmaceutical properties (e.g., solubility, dissolution rate, physical and chemical stability, and hygroscopicity) can be of high practical importance. Moreover, like salts, cocrystals can be used for the drug purification purposes (Bond *et al.* 2012).

The main difference between cocrystals and salts is the location of the proton between acid and base (Fig. 3). There is a common opinion that the salt will be formed by an acid and a base when the difference ΔpKa between $(pKa)_{base}$ and $(pKa)_{acid}$ is larger than 2 (known as the "rule of 2").

This criterion is traditionally used by drug development chemists as a cut-off rule to select the list of appropriate counterions for salt formation. In several studies, however, the crystal structures of salts and cocrystals with the number of coformers such that the difference ΔpKa varied between –9 and + 9 have been compared. It has been noticed that in some cases the location of the proton in the solid is affected not only by ΔpKa, but also by the temperature and crystalline environment (Childs *et al.* 2007; Childs and Zavorotko 2009). These studies demonstrate that it is not easy to make a division between salts and cocrystal by using only one criterion, such as the value of ΔpKa (Figure 4). In addition, the "rule of 2" does not predict the form, crystalline or amorphous, in which the salt of an acid and a base with ΔpKa > 2 will be obtained. This is the reason of why the concept of salt-cocrystal continuum has been introduced (Stahly 2007; Childs *et al.* 2007; Childs and Zavorotko 2009). According to this concept, the two classes are considered to belong to a single class of the salt-cocrystal continuum, in which pure salts and cocrystals are located at opposite edges. This implies that the "rule of 2" can be used only as an approximate dividing criterion when choosing an appropriate nomenclature, but not as an unconditional cutting-off criterion for choosing the components that might form a multi-component compound with a given drug candidate (Stahly 2007; Childs *et al.* 2007; Childs and Zavorotko 2009). It has been demonstrated that cocrystals, similarly to salts, can have considerably higher solubility than the neat drug substances, which is of prominent advantage for the drugs of BSC class II.

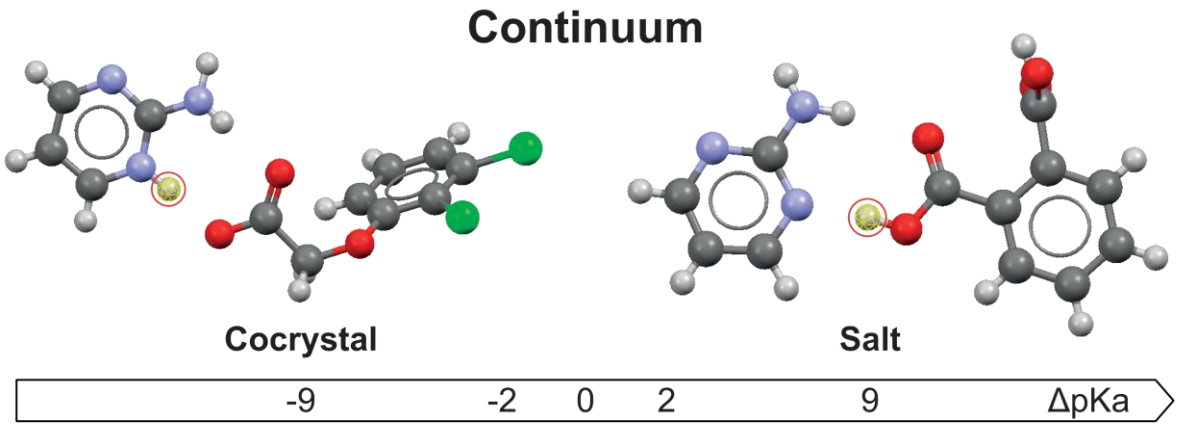



*Figure 3 Salt-cocrystal continuum.*

## 2.2 Solid-state form screening approaches

A very convenient, generic scheme for polymorph screening has been introduced by (Stahly 2007). This scheme can be extended to screening of all solid forms independently of such factors as the volumetric scale and total number of the samples, methods of crystallization and analysis, and the main target. The extended scheme is shown in Figure 4 and its detailed description is presented in the following subsections.

### 2.2.1 Characterization of materials in solid form screenings

The goal of the initial characterization of the material (step 1 in Figure 4) is to obtain basic knowledge of solid-state properties of the material that will be used in further steps of the screening. The starting material should be comprehensively characterized by using standard analytical techniques, such as powder x-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermogravimetry (TGA), thermomicroscopy, infrared (IR) spectroscopy, Raman spectroscopy, solid state magnetic resonance (ssNMR) spectroscopy, and gravimetric vapor sorption (GVS) analysis. It is also important to develop a method for appropriate verification of the chemical integrity of each new solid. This method can be based on solution NMR or high performance liquid chromatography (HPLC). Already during this characterization, an indication of possible polymorph or hydrate formation can be revealed (Cui and Yao 2008; Llinas and Goodman 2008; Brittain 2009; Tian et al. 2010). The obtained data will then be used in further characterization steps of the screening procedure. The same analytical techniques will be used also for the qualitative phase analysis of the samples (step 4, Fig.4) and characterization of each new form (step 6, Fig.4).

Since in majority of screenings the crystallization step is involved for generation of solid samples, it is necessary to evaluate the solubility of the material in the scope of organic solvents for both single component and multi-component screenings.

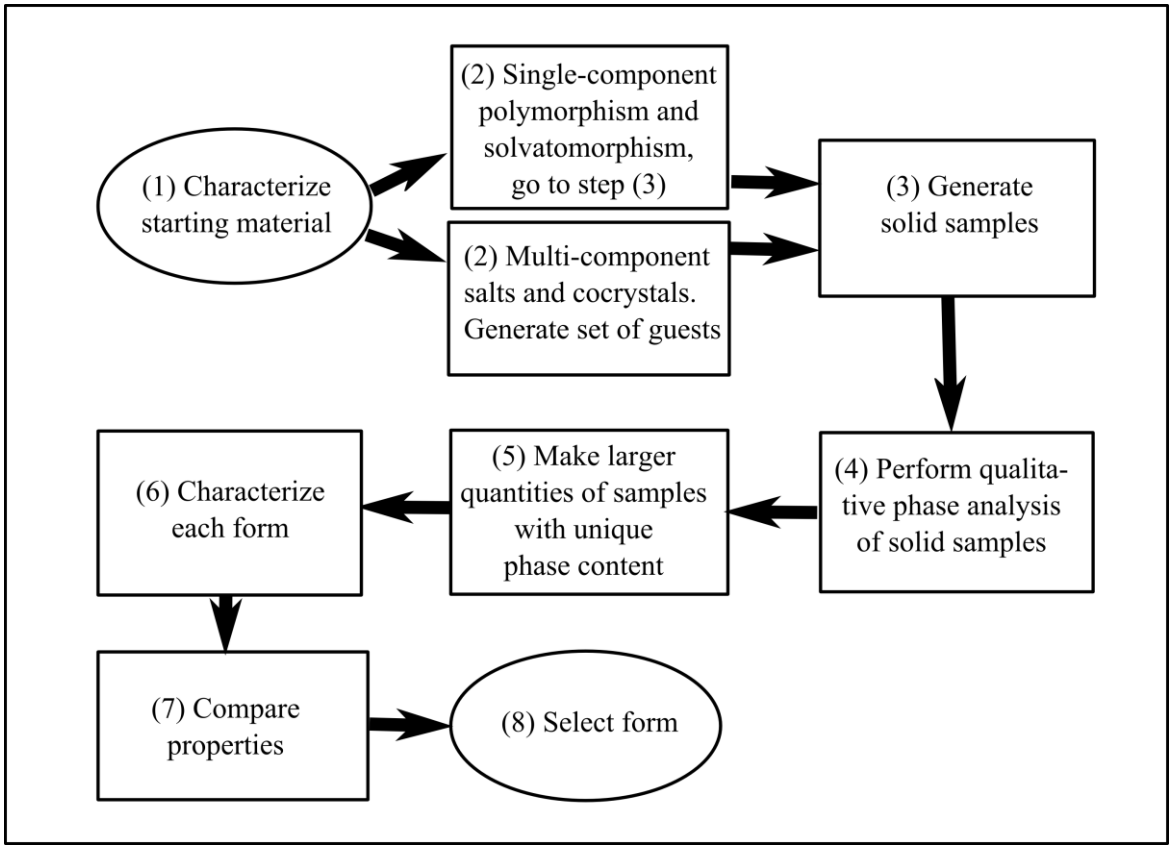



*Figure 4 Schematic presentation of solid-form screening and selection. Modified from (Stahly 2007).*

The technique might be chosen depending on the amount of the starting material available for the solubility assessment, for instance the well-plate technique, shake flask methods and gravimetrical methods can be used. The knowledge of the solubility in organic solvent is obligatory to establish the set of solvents for polymorph, solvate, salt or cocrystal screenings. It is also valuable to perform the solid state analysis of all solids remained in the result of the solubility analysis, and some new forms especially solvates might be discovered at this step (Byrn 1999; Alleso *et al.* 2008; Aaltonen *et al.* 2009; Brittain 2009; Bernstein 2011).

It is assumed that if the screening is performed for the multi-component solid forms the same procedure should be performed, if not done previously, for the other starting materials: acids or bases for salt screening and coformers for cocrystal screening if they are solids. Moreover, for the solvate, salt and cocrystal screenings, the NMR, HPLC and elementary analysis are valuable to reveal the stoichiometry of the components in the crystal. Also the coupling of TGA analysis with head space mass spectroscopy (TGA/MS)

is very useful for evaluation of stoichiometry and stability of the solvates and hydrochlorides.

Analysis of the single-crystal structure of the starting material and all the new obtained forms is of particular importance, since this structure explicitly reveals the conformations and packing configurations of the molecules in the crystal including possible hydrogen bonding schemes. In order to verify that the single crystal is the representative of the bulk, the PXRD pattern can be calculated for the single-crystal data and then compared with the experimentally obtained pattern of the bulk material. The information about the crystal packing is very important, since it can be used in the steps of selection of the most probable guest molecules that will form multi-component salts and cocrystals with the drug candidate molecule (Bernstein 2011). This will be considered in more detail in the following subsections.

### 2.2.2 Generation of solid forms

#### *Single-component polymorphism and solvatomorphism screening*

The approaches used in different laboratories for characterization of starting materials and obtained solid-state forms are more or less similar, but the methods of the solid sample generation can differ significantly (Hilfiker *et al.* 2006; Bernstein 2011;). In the majority of polymorph screenings, new solid-state forms are generated by crystallization from a diverse set of organic solvents, using different temperature profiles and concentrations (Stahly 2007). Hence, in spite of the fact that solvates are multi-component forms, their screening is convenient to consider in the same scope with screening of single-component compounds.

The crystallization techniques that do not use solvents are also utilized (Hilfiker et al. 2006; Hilfiker (ed.) 2006; Bernstein 2011;). These techniques include treatment of the starting material under specific thermodynamic conditions, aging in various vapors, and mechanical processing.

Due to a limited amount of substance available at the early discovery phase, most screening methodologies were adapted for using small-scale crystallization volumes and miniaturized-sample analysis techniques (Morissette *et al.* 2004; Stahly 2007; Bernstein 2011). As a result, in all these approaches, the crystallization conditions significantly differ from those used in ordinary organic synthesis processes. As a rule, they do not include molecular synthesis at all. The small-volume approaches have a tendency to produce metastable and amorphous forms, as well as the mixtures of them. Because of this the most stable form can remain undiscovered. Moreover, it should be emphasized that the first small-volume screening for polymorphs and solvates does not give a control over the manufacturing of these forms at bigger amounts of the material. Consequently, there is a practical need for development of cost-effective methods for evaluation of polymorphism

and solvatomorphism tendency. These methods must serve the discovery of the most stable solid form at ambient conditions.

### *Generation of solid forms of multi-component compounds: salts and cocrystals*

The progress from single-component screening to multi-component screening leads to emerging of additional challenges. In this case an additional step 2 that precedes sample generation (step 3, Fig. 4) must be used. In this step, a set of counterions and coformers shall be considered in view of formation of salts and cocrystals, respectively. It is recommended to use acids and bases as well as coformers that are proved to be safe for human (Stahl 2002). Considering the salt-cocrystal continuum concept, it is beneficial to choose the substances with various values of pKa and having diverse functionalities. It has been recently suggested that possible multi-component compounds can be theoretically predicted on the basis of supramolecular considerations. This topic will be separately reviewed in the upcoming sections (see section 2.3).

After the substances are selected for the screening and characterized, as described in section 2.2.1, the generation of solid-state forms can be started. According to numerous recent publications, there are no substantial differences in the approaches used for salt and cocrystal generation. Since both these approaches differ from the polymorph and solvate screening, care must be taken to prevent premature crystallization of one of the components in a pure form. This crystallization does not lead to complex or salt formation. There are several techniques used in multi-component screening for sample generation: (1) slow evaporation, (2) mechanochemical synthesis by cogrinding, (3) reaction cocrystallization, (4) slurry conversion, (5) high throughput (HT) screening, (6) comelting, and (7) cooling. The occurrence frequencies of these techniques in the literature are distributed as shown in Figure 5 (Sheikh *et al.* 2009).

All solvent based approaches, which are in the list, require selecting the best solvent or solvent mixture for the components which would result in the multi-component crystalline form of interest. In the slow evaporation technique the idea is to dissolve both components in solvent and let the solvent slowly evaporate and lead to supersaturation and subsequent crystallization.

The solvent or solvent mixture should be chosen by using the ternary phase diagram of the components and the solvent (Chiarella *et al.* 2007), since the diagram describes the three phase behavior of a system. In the slow evaporation, the pure cocrystal or salt will be successfully crystallized, if the solubilities of both components are close to each other. The ternary phase diagrams (Figure 6) depict the regions where the certain solid phase is in equilibrium with the solution: region 1 – all components are dissolved; region 2 – the component C is in solid form; in the region 3 – the cocrystal or salt is in equilibrium with solution; in the region 4 – the component N is in solid form; and in the regions 5 and 6 two solid phases are in equilibrium with solution: the cocrystal or salt and, correspondingly C and N. The points *a* and *a´* are the solubilities of C in methanol and water, respectively; and points *b* and *b´*- of component N in methanol and water. In the

Figure 6 the dashed straight line represents the drying line of the solvent upon evaporation. Methanol is chosen as a solvent for slow evaporation since the solubility of both components is similar (points *a* and *b* in the Figure 6 left). In this case the drying line of equimolar solution of C and N is within the stability region of the cocrystal (region 3,

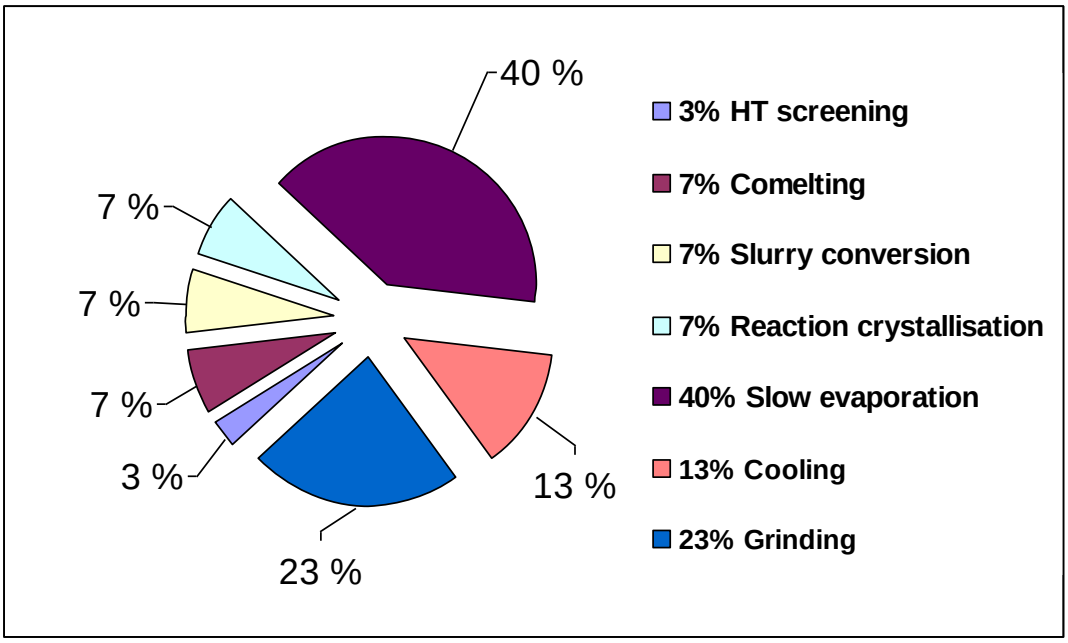



*Figure 5* *The cocrystal screening techniques occurrence in the literature, adopted from ( Sheikh et a. 2009).*

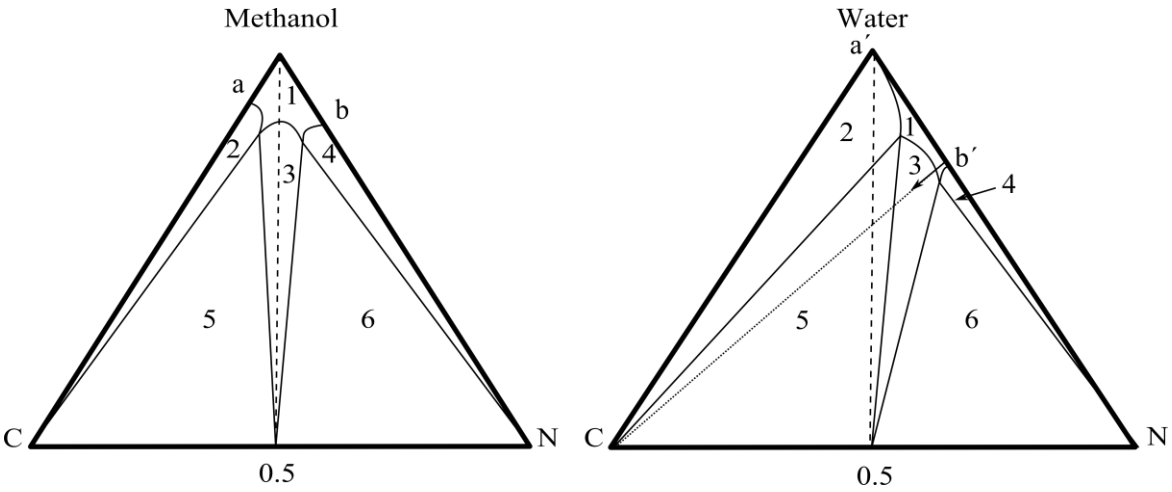



*Figure 6* *The cross section at T=25ºC of the ternary phase diagrams of cinnamic acid and nicotine amide with methanol (on the left) and with water (on the right).*

Figure 5 left) and it will crystallize in pure form. In the contrast, if the water would be used the drying line first meet the region where the component C is stable and it will be firstly obtained in solid form. Thus the resulting product will be the C and the cocrystal or

salt. Since the C will be crystallized first it is not guaranteed that the crystallization of the cocrystal will take place by kinetic reasons.

For the reaction crystallization method water might be a better choice. The saturated solution of the component N can be prepared (see *b´*– the composition of saturated solid solution) and the component C should be added in the solid form (along with dotted line starting from *b´* towards the C-corner in the diagram) until the crystallization of salt or cocrystal occurs (in the region 3 when the concentration of C will be within the end point of arrow). Therefore, in the reaction crystallization technique it is essential for a successful multi-component synthesis to select a solvent that gives a high solubility for the drug molecule and a low solubility for the salt or the cocrystal (Rodríguez-Hornedo *et al*. 2006; Jayasankar *et al*. 2009; Chadwick *et al*. 2009; Collman *et al*. 2013). The solution of the drug molecule can be prepared near the saturation point. Then, the guest substance should be added in a solid form until the desired salt or cocrystal will be crystallized from the solution.

In the slurry conversion synthesis the solubility should be taken into account also, since the slurry of both components should be prepared so that they will not be dissolved. This can be achieved by lyophilization or anti-solvent addition or cooling (Takata *et al*. 2008; Bučar *et al*. 2010).

The automated HT screening approaches, that include crystallization in tiny micro-wells (Kumar *et al*. 2007; Kojima *et al*. 2010) and the use of microfluidic platforms (Thorson *et al*. 2011; Goyal *et al*. 2012), have become very popular in the last years. In these approaches, in order to increase the chance of finding "hits", each compound is exposed to a diverse set of solvents, crystallization conditions, and techniques. In such screens, only small amounts of the material are generated and, therefore, a detailed characterization and understanding of the nature of the discovered form is impossible. Consequently, an additional scaling–up step is required to produce larger volumes of the compounds. Often, the scaling-up step fails to yield the same compound, in which case the findings of the first HT step have no practical value (Stahly 2007; Collman *et al*. 2013).

Successful obtaining of salts and cocrystals by using mechanochemistry methods, (2), has been recently reported in many scientific publications (Braga and Gepioni 2005; Friščić 2010; James *et al.* 2012). The success can be explained by the ternary phase diagrams (Chiarella *et al.* 2007). Since the dry solid components are mechanically grinded (with or without a very small drop of a solvent), the synthesis takes place in the close proximity of the stability region of the cocrystal or salt in the phase diagram. Consequently, the role of solubility of the components in the solvent is minimized. It should still not be underestimated. The method is ecologically sustainable, but it is limited from the industrial scale production point of view. Recently, however, an extrusion technique has been reported to give very promising results for scaling up the process (Medina *et al.* 2010).

The comelting approach, on the other hand, allows one to completely get rid of using solvents. In this case, it is enough to consider only the binary phase diagram. To accomplish the process, one has to mix the component in the stoichiometric proportion, heat the mixture to obtain the melt, let the system to reach equilibrium, and slowly cool the melt to obtain the salt or the cocrystal of interest. This approach, combined with

optical microscopy, was applied by Kofler already in 1943. Also Raman microscopy can be used for observation of crystallization dynamics when using this method (Davis *et al.* 2004; Berry *et al.* 2008). A serious limitation of the method is that it can be applied only if there is no chemical decomposition of the components during the melting.

The supercritical fluid technology (SCFT) has experienced a strong growth in the past decade, particularly in the field of materials processing, being more and more proposed as an alternative to many conventional solvent-based processes (Padrela *et al. 2009, 2010).* Using this alternative technology a group of scientists (Padrela *et al. 2009, 2010)* have obtained the cocrystals of indomethacin, theophylline, caffeine, sulfamethazine, aspirin and carbamazepin with saccharin.

The important pharmaceutical properties of all new solid-state forms obtained in the screening should be investigated and the best form should be selected for the development of the new drug candidate (steps (7) and (8) in Figure 3). This procedure will be considered in detail in section 2.4.

In the present study two most popular methods, the slow evaporation and the mechanochemistry synthesis, are compared in order to achieve practical understanding of the applicability of both techniques for industry.

## 2.3 Supramolecular chemistry and crystal engineering for selection of potential counterions and coformers

In order to select an appropriate set of counterions and coformers for the salt or cocrystal formation, one can use the methods of crystal engineering and supramolecular chemistry. The term ‘crystal engineering’ has been proposed by Schmidt in 1971 (Schmidt 1971) in connection with the photodimerisation reactions in crystalline cinnamic acids. Since then, crystal engineering has become a well established branch of science that in fact includes also some aspects of the solid-state supramolecular chemistry. Perhaps one of the most accurate definitions, proposed by Gautam Radhakrishna Desiraju in 1988, defines crystal engineering as "the understanding of intermolecular interactions in the context of crystal packing and the utilization of such understanding in the design of new solids with desired physical and chemical properties" (Desiraju 2007; 2009).

The main interactions responsible for the salt formation are of ionic nature. There is a common opinion that the base and acid will form a salt if the difference between their pKa values will be larger than 2. Thus, if the value of ΔpKa of a drug-candidate molecule is calculated or determined experimentally, a set of appropriate acids or bases can be formed by considering their pKa values. This approach, however, does not guarantee that all salts will be obtained in a crystalline form (Trivedi *et al.* 2003). Moreover, if the drug molecule contains several protonation sites, it is not always clear which site will be protonated. Finally, as it was described in subsection 2.1.4, in those cases when the $0 < \Delta pKa < 2$ there is no guarantee that there will be proton transfer in solid state. Thus the guidelines described in the following paragraph are similarly applicable to salts (Mei and Wolf 2004; Ong 2011).

In non-ionic crystals, the crystallization is guided by less predictable and weaker interactions, such as hydrogen bonding, π-π bonding of aromatic rings, van-der-Waals bonding and halogen bonds. For favored hydrogen bond arrangements in non-ionic crystals, certain guiding principles (based on scrupulous analysis of hydrogen bonds and packing motifs) have been developed by Donohue 1959 and Etter 1990 (Byrn 1999) These principles include the following guiding rules: (a) all acidic hydrogens available in a molecule will be used in hydrogen bonding in the crystal structure of the new compound, (b) intramolecular hydrogen bonds that form a six-membered ring will occur in preference to intermolecular hydrogen bonds; (c) the best proton donors and acceptors remaining after formation of intramolecular hydrogen bonds form intermolecular hydrogen bonds to one another; (d) all good acceptors will be used in hydrogen bonding when there are available hydrogen-bond donors, and (e) the best hydrogen-bond donor and the best hydrogen-bond acceptor will preferentially form hydrogen bonds to one another. Additional rules for specific classes of functional groups have been also determined. Etter also mentioned various reasons that can make these principles inapplicable (Etter 1990; 1991). These reasons include the presence of multiple competitive hydrogen-bond sites, conformational freedom, steric hindrances, and competing dipolar or ionic forces.

The Cambridge Structure Database that contains empirical structures of a half a million of organic crystals gives an additional opportunity for searching for the most common functional groups and the information on how they can be connected in a molecular association (Chisholm *et al.* 2006; Laszlo 2009). These types of non-covalent connections between functional groups are supramolecular synthons that are analogous to the covalent bonds in synthetic chemistry (Vishweshwar et al. 2006). Therefore the guest molecules having the complementary favorable functional groups can be chosen as the most probable candidates for screening (Laszlo 2009). The example of the supramolecular considerations for selecting the most probable host-guest pairs is described below. For instance, according to (Vishweshwar et al. 2006), carboxylic acid groups exist in 30 of 100 top-selling prescription drugs in USA and they are the most frequently studied functional groups in supramolecular chemistry. Their hydrogen-bond donor and acceptor sites make a supramolecular homosynthon I (see Figure 7) favorable. However, formation of this synthon is unlikely in competitive situations. A CSD study on carboxyl donors indicated that carboxylic acid–pyridine interactions through O-H…N hydrogen bonding, synthon II, is more favored than synthon I (Vishweshwar et al. 2006). Indeed, the synthon II is a reliable supramolecular heterosynthon that has been widely exploited in crystal engineering. Furthermore, the synthon II is energetically favored over the synthon I and the acid-amide supramolecular heterosynthon IV is favored over synthons I and III (Vishweshwar et al. 2006). These examples show those types of considerations that should be applied in the design of co-crystals, since the use of the robust supramolecular heterosynthons are probably the most reliable and rational route to obtaining co-crystals (Aakeröy and Salmon 2005; Miroshnyk *et al.* 2009; Delori *et al.2013*). Moreover, the ionic synthones could be used for design of salts (Mei and Wolf 2004; Ong 2011).

It is not clear, however, whether these simple considerations can be applied if the functional groups of the drug candidate contain several good acceptors and competitive donors. Analysis of the last-decade publications shows that very often, formation of a co-

crystal is difficult, if not impossible, to predict, especially when the molecules have complicated structures (Desigaju 2012). Co-crystallization of cis-itraconazole with a series of 1,4-dicarboxylic acids can be mentioned as an example.

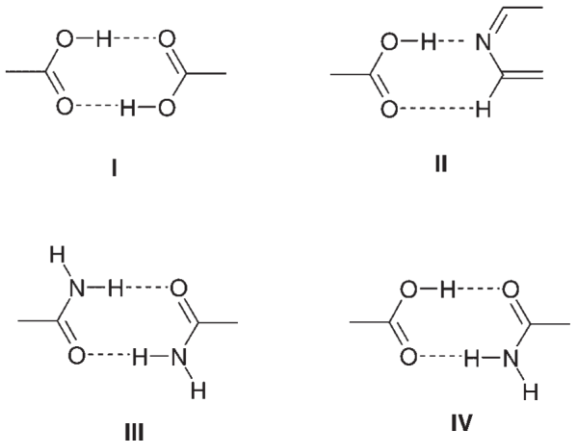



*Figure 7 Examples of the most popular synthons. Adopted from (Vishweshwar et al. 2006).*

It is remarkable that the interaction between succinic acid and the strongest basic group of itraconazole (piperazine moiety) was not present in the co-crystal structure (Remenar *et al.* 2003; Blagden *et al*. 2007). Instead, the COOH-group of succinic acid was found to be connected to a 1,2,4-triazole group, which is not an expected result.

Crystal engineering and supramolecular synthesis of complex molecules are currently very active areas of theoretical and experimental investigations. Moreover, the reversible molecular interactions that hold together the API and the co-crystal former (or co-former) have the same nature (i.e., H bond, Van der Waals interaction, metal coordination) as those that take place between the API and its biological target. This makes the co-crystallization a general approach on the pharmaceutical arena (Desigaju 2012).

## 2.4 Selection based on evaluation of pharmaceutical properties

The main goal in performing solid-state screening is to find a form that will have optimal properties in view of development of a new drug candidate (Stahly 2007; Byrn 2012). Therefore, the properties of the forms found in the screening must be carefully studied and compared with each other. The most critical properties that must be improved should be selected as a main target to tackle (Gould 1986; Newman et al. 2010). This can be done on the basis of the in-silico predicted physical and chemical properties of the new drug candidate and the initial characterization results of the first solid batches of the material. The new drug candidate may have poor solubility and bioavailability, chemical and/or physical stability and crystallinity or high hygroscopicity (Byrn 2012). Table 2 shows (a) a list of important solid-state properties to be addressed in the research, (b) the associated characterization methods, and (c) a description of the properties of an ideal candidate for

the development of an oral dosage form. The table can be used as a guide for selection of the most promising solid-state forms for new drugs.

**Table 2.** *Properties of the solid-state forms for selection of the optimal one.*

| Property | Method | Ideal properties for development of oral dosage form |
|---|---|---|
| Solubility | Shake flask method. Solubility in biorelevant media or in buffer solution | Proposed dose should be soluble in 250 ml biorelevant media in pH range 1.2-7 |
| Dissolution rate | IDR using Wood's apparatus in biorelevant media or buffer solution | IDR > 1mg/cm$^2$/min in pH range 1.2-7 |
| Bioavailability | Pharmacokinetic study on animals (rat, dog, monkey). Measurement of AUC or $C_{max}$, depending on the product target. | Bioequivalent or better than i. v. solution. |
| Crystallinity | PXRD | Crystalline. The absence of amorphous content |
| Melting point | DSC, thermomicroscopy | Melting point preferably >100ºC |
| Hygroscopicity | GVS | Not hygroscopic in the RH range 10-90%. If hygroscopic, than controllable formation of stable hydrate |
| Physical stability | GVS, DSC, non-ambient PXRD, solid-state spectroscopy (Raman, IR, NIR, Teraherz, NMR) after stressed conditions at 60ºC/100% RH for 2 weeks | No phase transitions induced by moisture or temperature changes.<br>No disproportionation of the salt or cocrystal. |
| Polymorphism | Batch-to-batch PXRD, DSC, recrystallization from 4-5 solvents, grinding experiments | No or low polymorphism tendency |
| Chemical stability | Degradation studies performed under stressed conditions for 2 weeks | Stable in the solid form under stressed conditions |
| Crystallization process | Scaling-up of crystallization. Measuring of the yield. | Scalable process with high yield using Class 3 solvents |

### *Solubility/dissolution rate and bioavailability*

The need for enhancement of aqueous solubility of new drug candidates is commonly recognized as a crucial step in the pharmaceutical sciences. In our studies, the solubility enhancement is considered as one of the most important priority factors for the form selection. The solubility and the dissolution rate of the drug can be tailored by prudent selection of the salt. For example, in this way, the solubility can be enhanced by two

orders of magnitude (Stahl 2002; Serajuddin 2007) and still, if necessary, it can be significantly reduced by selecting hydrophobic, fatty or bulky counterions (e.g. laurate, pamoate, resinate) (Aungst and Hussain 1992). The solubility reduction can be used in the controlled and sustain release development.

The solubility and dissolution rate of very weak bases or acids, as well as of non-ionic molecules, can also be enhanced by formation of cocrystals (Remenar *et al*. 2003; McNamara *et al*. 2006; Wassvik *et al*. 2006; Stahly 2007; Bak *et al*. 2008; Good *et al*. 2009; Babu and Nangia 2011; Gao and Zang 2011; Smith *et al*. 2011; Stephenson *et al*. 2011; Elder *et al*. 2012; Chadha *et al*. 2012; Weyna *et al*. 2012; Xu *et al*. 2012; Brittain 2013; Steed 2013; Thakuria *et al*. 2013). To my knowledge, cocrystals have not been used to reduce drug solubility. However, according to (Elder *et al*. 2012), formation of cocrystals can also lead to a solubility reduction.

The solubility of thermodynamically less stable polymorphs is usually the same or higher than that of more stable polymorphs (Llinas 2007). However, the solubility enhancement through crystallization of different polymorphic forms is not as high as by means of formation of salts. A comprehensive review on the degree of solubility enhancement by high-energy polymorphs is given in (Pudipeddi and Serajuddin 2005). According to this paper, less than 5 fold enhancement was achieved in the majority of cases, with an exception for premafloxacin (23 folds). The reason for the limited spread in the solubility ratios can be in a rather small energy difference of the molecular-crystal modifications that often results from a packing or a conformational difference (Pudipeddi and Serajuddin 2005).

As a rule, formation of pharmaceutically acceptable solvates gives a higher solubility enhancement, 10-15 folds. Solvates, however, have a propensity to gradually loose the solvent of crystallization, which makes them less attractive in view of practical applications. On the other hand, formation of anhydrates instead of hydrates, if they can be made stable at normal conditions, is always of high practical interest (Pudipeddi and Serajuddin 2005). The hydrates, being more stable in water than anhydrates, are known to have a lower aqueous solubility (Grant 1990; Pudipeddi and Serajuddin 2005).

The enhancement of the solubility and the dissolution rate should be ensured to translate into an increased in-vivo exposure, since there are risks of the salt disproportionation and a common-ion effect. As an example, an increased bioavalability of a low soluble drug candidate was achieved by cocrystal formation with saccharine (Bak *et al*. 2008).

### *Hygroscopicity*

Salts with high solubility have been noticed to show also a high hygroscopicity, which can be an issue for salts of strong acidic (particularly di-HCl) and basic counter ions, such as Na and K. High hygroscopicity, however, can induce problems in the pharmaceutical processing and storage of both the API and the drug product (Morris et al., 1994). This problem should definitely be addressed in the future pharmaceutical research.

### *Melting point*

Melting point is an important characteristic of all solids. In view of pharmaceutics, high melting points are usually considered to be beneficial. However, they can contribute to poor solubility and be as problematic as low melting points that are known to hinder the processing, drying, and stability of the material. For salts, the enhancement of solubility is obtained primarily due to the ionization effect. For cocrystals and polymorphs, on the other hand, the role of the crystalline lattice energy and, consequently, the melting point are of particular importance. The correlation between the solubility and the melting points of the cocrystals is currently a subject of thorough investigations (Chu and Yalkowsky 2009; Elder and Holm 2012). In (Stanton and Bak 2008), for example, a good correlation between the melting points of the coformer and the cocrystal has been found, which provided the possibility to tailor the properties of the cocrystal by selection of the coformer. Simultaneously, the authors revealed less correlation between the melting point and the solubility of the cocrystals. Obviously the melting point is not the only factor affecting the solubility. For instance the hydrophilicity of the coformer can also play an important role (Elder *et al.* 2012).

### *Polymorphism and solvatomorphism tendency*

As already mentioned in subsection 2.1.3, both single- and multi-component substances can exhibit polymorphism. A comprehensive statistical analysis of empirical data on polymorphism tendency is presented in (Stahly 2007). The data in question is based on the 245 polymorph screens conducted by SSCI. The analysis shows that single compounds are more frequently polymorphic than salts (55% compared to 39%), but the salts more frequently exist as hydrates (48% compared to 30%). This is an expected result based on the known affinity of water to bind to ionic sites. Many organic molecules are able to crystallize into many polymorphic forms, although there are examples of molecules that do not have this ability, for instance ibuprofen. The ability of the organic substances to crystallize in different forms cannot usually be predicted. Therefore, it must be found through experiments (Stahly 2007). Because of the lack of a representative amount of experimental data, analytical study of the polymorphism tendency of cocrystals is impossible at the moment and therefore it must be conducted in the future. The importance of this research is discussed in (Eddleston *et al.* 2013), where the authors study the polymorphism tendency of a phenazine:mesaconic acid (1:1) cocrystal. Discovery of three polymorphs, including one hydrate and one DMSO solvate, of the cocrystal in the screening is reported.

The propensity for the polymorphism can be evaluated using a small amount of material, say, 100-200 mg. Therefore, to enable the best form selection for the development, the polymorphism tendency evaluation should be done in the later stages of drug discovery. It is important to emphasize, that at these stages, it is far more important to discover the most stable form of the new drug candidate than to discover its all possible polymorphic forms. Focusing the screening on finding this most stable form can ensure

the scalability of the crystallization process and enable the proper form selection for the development (Stahly 2007).

### *Physical stability*

As mentioned above, the selected solid-state form must show an acceptable physical stability both at the crystallization conditions and at normal laboratory conditions in order to enable the scalability of the crystallization process and the long-term shelf-life of the API and the oral drug form. The physical stability for different polymorphs of a compound is connected to such thermodynamic properties as monotropicity and enantiotropicity which therefore must also be determined (Stahly 2007, Newman *et al.* 2010; Byrn and Henck 2012). Furthermore, the physical stability of the discovered solvates, including hydrates, should be tested at stressed conditions (Stahly 2007; Newman *et al.* 2010; Byrn and Henck 2012), and finally, the disproportionation tendency of the salts or cocrystals, if such are discovered, must be evaluated. The propensity for the disproportionation has been reported for hydrochlorides and other salts of very weak bases. It is indeed a much undesired property that can lead to a significant reduction of the solubility and the dissolution rate (Guerrieri and Taylor 2009; Stephenson *et al.* 2011).

### *Crystallinity and crystallization process*

The main advantage of the crystalline solid-state forms over the amorphous ones is that the former can have high solubility and simultaneously be more stable both physically and chemically. Because of this, the crystallinity of the polymorphs, solvates, salts or cocrystals must be controlled and only the crystalline forms must be selected for further development, unless the amorphous form is what is desired for some other reason.

The formation of a salt or a cocrystal can often help to crystallize a substance that is difficult or impossible to crystallize otherwise (Wouters and Quéré 2012). Furthermore, the formation of salts and cocrystals has been shown to lead to an effective purification of the sample from synthesis impurities and from stereomers and enantiomers (His *et al.* 2012; Billot *et al.* 2013). Naturally, the forms that can enable both crystallization and purification are of great interest for medicinal chemists.

### *Chemical stability*

New solid-state forms of a given drug candidate must be demonstrated to have at least the same chemical stability as the neat material. Many drug molecules, however, are susceptible to decompose, which can complicate or even stop the drug development. Forming of a salt can be a very efficient way to enhance the chemical stability of the drug against the decomposition (Stahl 2002). Also cocrystals are reported to offer this

possibility. As an example, a 1:1 cocrystal of nitrofurantoin-4-hydroxybenzoic acid has been shown to have a superior photostability compared to the neat nitrofurantoin (Vangala *et al.* 2011).

### *Mechanical properties*

Different solid-state forms due to different internal as well as external structureswill have different mechanical properties, such as flowability and compressibility. Consequently they are of importance for the manufacturing process of the drug product. The scope of the mechanical properties is important to consider at the later development stages of the drug development process and they are excluded from the scope of the present study.

# 3 Aims of the Study

The aim of this study was to develop means for expanding diversity of developable solid-state forms of weakly basic drug molecules to be applied in pharmaceutical sciences.

The specific aims of the study were:

(1) To find the most stable form of hydrochloride salt of a new drug candidate in order to enable the effective pharmaceutical development of the oral dosage form.

(2) To assess the feasibility of molecular modeling tools for coformer selection.

(3) To develop an effective screening approach that can be used in industry for salt and cocrystal screening.

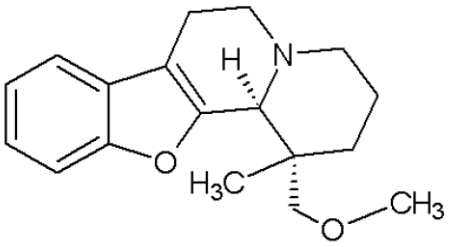
(4) To conduct the comparative study of the most critical pharmaceutical properties of new salts and cocrystals of itraconazole.

# 4 Experimental

Detailed description of the utilized experimental approaches can be found in the original publications (I-IV).

## 4.1 Materials

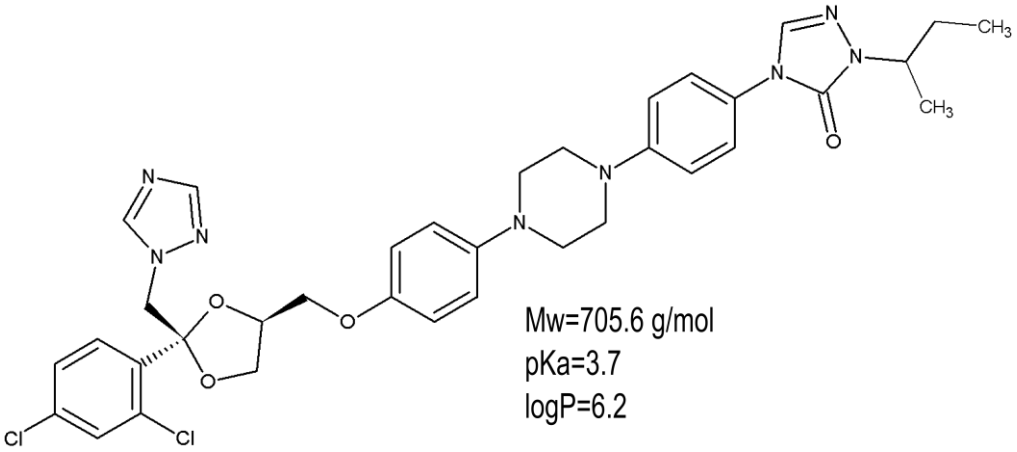
The basic drug substance used in the Publication I was a new drug candidate ORM10921, IUPAC name [1R*,12bR*)-(-)-1,3,4,6,7,12b-Hexahydro-1-methoxymethyl-1-methyl-2Hbenzofuro [2,3-a]quinolizine] (Figure 8). Its hydrochloric salt were synthesized by Orion Pharma, Finland. The absolute configuration was assigned by optical rotation and later by single crystal X-ray diffraction. The optical purity of the material was >97%.




MW=285.4 g/mol
pKa=7.3
logP=4.4

*Figure 8 Chemical structure of the investigational drug, ORM10921.*

The weak base used in the rest of Publications (II-IV) was itraconazole (ITZ, purity 99.7% see structure in figure 9). The material was purchased from Apotecnia S.A.




Mw=705.6 g/mol
pKa=3.7
logP=6.2

*Figure 9 Chemical structure of itraconazole.*

Dicarboxylic acids (C2-C10) used in Publications II, III and IV, which are oxalic acid anhydrate, malonic, succinic, glutaric, adipic, pimelic, suberic, azelaic, and sebatic acids (ACS reagent > 99%) (Figure 10) was purchased from Sigma-Aldrich. Concentrated 37% hydrochloric acid water solution (Sigma-Aldrich Co.) and HCl gas (Aga Linde Group, purity 99.995%) were used as received. The analytical grade solvents used for the studies were obtained from the commercial sources.

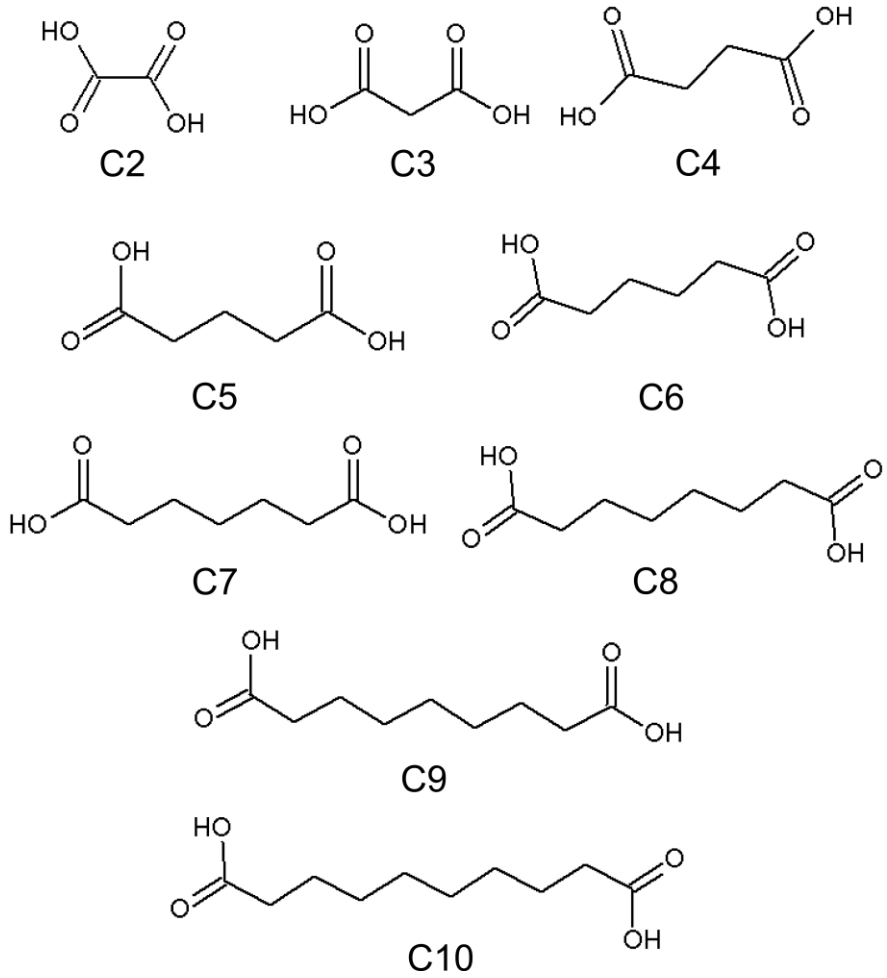



*Figure 10 Chemical structure of the dicarboxylic acid of growing carbon chain length (C2-C10) used in the study. Oxalic (C2), malonic (C3), succinic (C4), glutaric (C5), adipic (C6), pimelic (C7), suberic (C8), azelaic (C9) and sebacic (C10) acids.*

## 4.2 Preparation of new solid state forms

### 4.2.1 Hydrochloric salts of ORM10921

The final crystallization of the synthesis process consisted of dissolving of the ORM10921 free base in ethyl acetate or a mixture of ethyl acetate with another solvent. Then 10%

solution of HCl in ethyl acetate was slowly added with a constant stirring rate. After accomplishing the salt precipitation, the slurry was cooled in an ice bath for 3 h, filtered, and washed in ethyl acetate with subsequent drying in vacuum at elevated temperature.

### 4.2.2 Hydrochloride salts of itraconazole

*Gas bubbling (Method A)*

Itraconazole was added to acetone. The anhydrous HCl gas was bubbled slowly into the mixture. After the hydrochloride salt was precipitated the final product was collected by filtration, washed with acetone and dried in vacuum.

*Salting out from solution (Method B)*

Itraconazole was added to acetone. Then the concentrated (37%) water solution of HCl was added to the mixture. The slurry was heated to 50 ºC. The clear separation of transparent liquid was followed by precipitation of white flakes. The mixture was allowed to stabilize overnight at room temperature. Thereafter the product was collected by filtration, washed with acetone and dried in vacuum.

### 4.2.3 Cocrystals of itraconazole and dicarboxylic acids

*Mechanochemical synthesis by solvent assisted grinding*

The mechanochemical synthesis we used in the study proceeds as follows. The neat itraconazole and acids are weighted in desired ratios to obtain 1 g samples. The grinding experiments are performed using a planetary ball mill (Pulverisette 6, Fritsch GmbH, Germany) in a stainless steel grinding bowl of 80-mL volume with four grinding balls. The synthesis is assisted with acetone (200 µL). The resulting powder was removed from the bowl into a glass vial and dried in vacuum.

*Slow evaporation*

Stock solutions of pure itraconazole in chloroform and a dicarboxylic acid in tertrahydrofuran (THF) and chloroform (CLF), respectively, were mixed in glass flask to obtain 1:1 and 2:1 molar mixtures of itraconazole and acid, correspondingly. An excess solvent was then added, if needed, to form a 1:1 (CLF:THF) volume ratio. The prepared solutions were heated with continuous stirring to ensure complete dissolution of the solids.

Thereafter, the bottles were sealed with a perforated film, through which the solvent slowly evaporated. After appearance of the first crystals, the film was removed to speed up the evaporation. The final drying, after the complete evaporation of the solvent, was performed in a vacuum oven.

### 4.2.4 Growing of single crystals of studied forms

A good quality single crystal of the ORM10921·HCl hemihydrate was selected from the 1g batch (B6 in Publication I) crystallized by the described synthesis process where acetate/water (90/10 v/v) was used as the crystallization solvent.

*Single crystals of the neat itraconazole*

The single crystals of itraconazole were obtained by recrystallization of commercial itraconazole in dimethyl sulfoxide. In a clean and dry test tube (10 mL) itraconazole (100 mg) was taken and added dimethyl sulfoxide (2.0 mL) and the suspension was heated until a clear solution was obtained. The resulted solution was allowed to attain room temperature. The crystalline precipitate appeared after 12 h and was collected directly from the test tube.

*Itraconazole-succinic acid (2:1) cocrystal*

To a mixture of itraconazole (200 mg, 0.283 mmol) and succinic acid (16.8 mg, 0.142 mmol) taken in a 25 mL r.b. flask, a mixture of solvents (15 mL) consisting of 1,2-dichloroethane, ethyl acetate and 1,4- dioxane (10/2/1 v/v/v) was added. The resulted mixture was heated until a clear solution was formed. The solution was allowed to attain room temperature and after 8 h the crystalline solid was filtered.

## 4.3 Analytical methods

### 4.3.1 Solid-state characterization and pharmaceutical properties of new forms

*Qualitative phase analysis by x-ray powder diffraction (PXRD)*

For qualitative phase analysis the conventional X-ray powder diffraction method was used. Philips X'Pert PRO multipurpose θ-θ diffractometer equipped with the RTMS (Real

Time Multiple Strip) detector. Filtered Kα radiation from Cu tube at 40 mA and 45 kV was used. The qualitative phase analysis was carried out by comparing the experimental PXRD patterns with patterns of known solid forms found from Cambridge Structure Database (CSD), Powder Diffraction File (PDF)/2 and PDF/4 of the International Centre for Diffraction Data® (ICDD) as well as by the manual comparison of patterns found in literature.

### *Raman spectroscopy*

New forms were studied by Raman spectroscopy as a complementary characterization technique giving deeper insight into the structural changes caused by cocrystal formation on molecular level. Raman spectra were collected using a dispersive Raman microscopy (inVia, Renishaw) using a 785 nm diode laser, and 20-50x magnification objective lenses.

### *Thermal analysis (DSC,TGA)*

The differential scanning calorimetry (DSC) and thermal gravimetrical analysis (TGA) were conducted as supporting methods for the solid-state characterization. Melting behavior was analyzed by differential scanning calorimeter (DSC821e, Mettler Toledo AG, Switzerland). The scans were performed, heating the sample from 25 to 240 ºC with a heating rate of 10 ºC/min in nitrogen atmosphere. Loss of weight was monitored with a thermobalance (TGA 851e, Mettler Toledo AG, Switzerland). Samples were analyzed at a heating rate of 10ºC/min under nitrogen purge.

### *Hygroscopicity and moisture sorption-desorption isotherms (GVS)*

The moisture sorption-desorption isotherms were measured by the humidity and temperature controlled microbalance system, Dynamic Vapour Sorption (DVS 1, Surface Management Systems Ltd, United Kingdom). The moisture sorption-desorption behavior of the samples (~10 mg) was determined by continuously measuring the weight change of the samples with two successive cycles of relative humidity (RH), each one starting from 0% to 90%-95% and backward to 0%, using 10% RH steps. After the experiments the qualitative phase analysis was performed by PXRD in order to detect possible phase transition caused by the high humidity.

### *Intrinsic dissolution rate (IDR)*

IDR testing of all solid state forms of ITZ were performed using the dissolution device Sotax AT7 (Sotax AG, Switzerland) equipped with rotating disc (Wood's apparatus). The solid powder was compressed in a die against a steel plate to yield a disk of known surface

area (0.5 $cm^2$). Before and after the IDR measurements, the crystallinity and phase composition of the compacts was verified by PXRD. The dissolution studies (n=3) were carried out in 500 mL medium at pH 1.2 (hydrochloride acid buffer, Ph. Eur. 6.8) and 37 ºC. For each time point, 1 mL of dissolution medium was collected during the experiments and replaced with 1 mL of fresh pre-warmed (37 ºC) medium.

### 4.3.2 Structure elucidation and purity verification

*Nuclear magnetic resonance (NMR)*

The solid state NMR 13C CPMAS spectra were recorded on a Bruker AV400 (spectrometer equipped with a 4 mm standard bore CPMAS probe head whose X channel was tuned to 100.62 MHz for 13C. The 13C CPMAS NMR was carried out for all samples under Hartmann–Hahn conditions with TPPM decoupling. The 13C CPMAS NMR chemical shifts are referenced to the C=O signal of glycine at 176.3 ppm and 15N CPMAS chemical shifts were referenced to the glycine signal at -345.25 ppm.

The solution NMR spectra were collected on a Bruker Avance III 600 MHz equipped with 1.7 mm TCI inverse-cryoprobe with z-gradient. Experiments were conducted at temperature of 300 K. The verification of a proposed structure was accomplished by using 1H, 13 C{H}, COSY, HSQC, HMBC and ROESY experiments. Proton and carbon chemical shifts were calibrated to TMS (δ=0.0) or residual DMSO-d6 signal (δC = 39.52 ppm). Acquired NMR data were processed by TopSpin 2.1 (Bruker, version 2.1), ACD 1D, 2D Manager and ACD C13 Predictor (Advanced Chemistry Development, Inc., ACDLabs version 11.02) both Perch software (Perch Solutions Ltd., Version 2009.1 SA)

*High performance liquid chromatography (HPLC)*

The purity of ORM10921·HCl hemihydrate was determined by HPLC using Agilent 1100 series (Agilent Technologies, Germany) chromatograph consisting of binary pump, a column compartment, a multi-wavelength detector UV-VIS, a well plate autosampler and a degasser. The separation of ORM10921 was achieved by using an XTerra MS C18, 250 x 4.6 mm, 5 µm column at a flow rate 1.0 mL/min. The mobile phase during determination (λ= 250 nm) was acetonitrile and 10 mM ammonium bicarbonate water solution at pH 9. Sample injection volumes of 10 µL were used for analysis.

The itraconazole content of the samples from IDR and the purity of the solid state forms were determined by HPLC using an Agilent 1100 series (Agilent Technologies, Germany) chromatograph consisting of a binary pump, a column compartment, a multi-wavelength detector, a well plate autosampler and a degasser. The separation of itraconazole was achieved by using a Gemini C18 column and a flow rate of 1 mL/min.

The mobile phase during determination (λ = 261 nm) consisted of acetonitrile and 0.1% trifluoroacetic acid pH 2.0. Sample injection volumes of 20 µL were used for analysis.

### *Elemental analysis (EA)*

EA of the samples were performed by standard combustion method (Vario Micro cube Elementar Analysensysteme GmbH, Germany). Samples were fed automatically into a combustion zone. Combustion gases were sent to a catalytic post combustion zone and then to a reduction zone. The formed analyte gases $N_2$, $H_2O$, $CO_2$ and $SO_2$ carried by He gas were sequentially separated by a temperature programmable desorption (TPD) and quantitatively determined on a thermo-conductivity detector (TCD).

### *X-ray single-crystal analysis*

*Single-crystal diffraction analysis performed for the ORM10921 hydrochloride hemihydrate*

The single-crystal X-ray diffraction data were collected using a Bruker Nonius (1998) single crystal X-ray diffractometer. The cell refinement and data reduction were performed with DENZO and SCALEPACK software. The software to solve the structure was SHELX97 and to refine the structure SHELS97.

*Single-crystal diffraction analysis performed for the neat itraconazole and itraconazole-succinic acid cocrystal*

Good quality plate-shaped crystals were subjected to the single crystal X-ray diffraction analysis. In order to examine the validity of the suggested disorder model (proved to be similar in all crystals), several single crystals were used for the structure analysis. The data collections were carried out both at -150.0 ± 0.1 and at 0.0 ± 0.1 ° (cooled by Oxford Cryostream) using Agilent Supernova dual wavelength diffractometer with two micro-focus X-ray sources (Cu and Mo) of which multilayer optics monochromatized CuKα radiation was applied (λ = 1.54184 Å; 50 kV, 0.8 mA). The data acquisition (Atlas detector), reduction, multi-scan and analytical face-index based absorption corrections were all made by program CrysalisPro. The crystal structure was solved by ShelXS2 implemented in program Olex2 (v 1.2 ) and refined on F2 by full matrix least squares techniques (ShelXL) using anisotropic displacement parameters for all non–hydrogen atoms.

# 5 Results and Discussion

## 5.1 Polymorphism and solvatomorphism tendency evaluation of the hydrochloride salt of a new drug candidate, I

ORM10921, a promising new drug candidate, has exhibited efficacy in rodent models predicting antipsychotic and antidepressant activity. This molecule is a weak base (pKa = 7.4) that shows a low water solubility (7.5 μg/mL in phosphate buffer at pH = 7.4). To enhance the solubility of the ORM10921 base, a salt formation approach was employed, and a traditional hydrochloride (HCl) salt was synthesized. The main focus of the study reported in Publication I was on evaluation of the polymorphism and solvatomorphism tendency and stability of ORM10921·HCl.

### 5.1.1 Rational polymorphism screening approach based on the properties of the initial material, I

According to the PXRD analysis (I, Fig. 4), the amorphous form was found to be a predominant solid phase for the initial three batches of the material. Moreover, the moisture-induced crystallization of this amorphous material was revealed at 55% RH. The crystallization was manifested by a sudden weight drop during the first moisture sorption cycle performed by GVS (I, Fig. 4A). The fact of crystallization was further verified by the PXRD pattern that exhibited sharp diffraction peaks and the absence of the amorphous halo (I, Fig. 4B). The TGA analysis of the sample after GVS revealed the 2:1 stoichiometry of ORM10921·HCl:$H_2O$. Analyzing the obtained results, it has been hypothesized that, upon exposure to moisture, the amorphous material tends to crystallize into a more stable crystalline form, a hemihydrate (ORM10921 HCl·1/2 $H_2O$; hereafter denoted as HH).

These observations confirmed well-known propensity of hydrochloride salts to form hydrates. Thus, we have chosen a rational approach for the fast initial polymorphism and solvatomorphism tendency evaluation tailored specially for this case. Our polymorphism screening approach is based on systematic examination of solid-state properties of subsequentially synthesized batches at the real-process scale. This approach is schematically presented in Figure 11. Briefly, physical characterization of each fresh batch of a new substance is performed by means of standard methods, such as PXRD, DSC and TGA. This characterization gives a feedback for optimization of the crystallization parameters used to crystallize the next batch. Simultaneously, in order to evaluate the physical stability at high relative humidity, every single batch is analyzed by the GVS method combined with PXRD and TGA analysis that were performed before and after GVS. The final crystallization step of the synthesis is adjusted until a stable solid form can be reproducibly crystallized. The key variables were the water content of the crystallization solvent and the drying conditions.

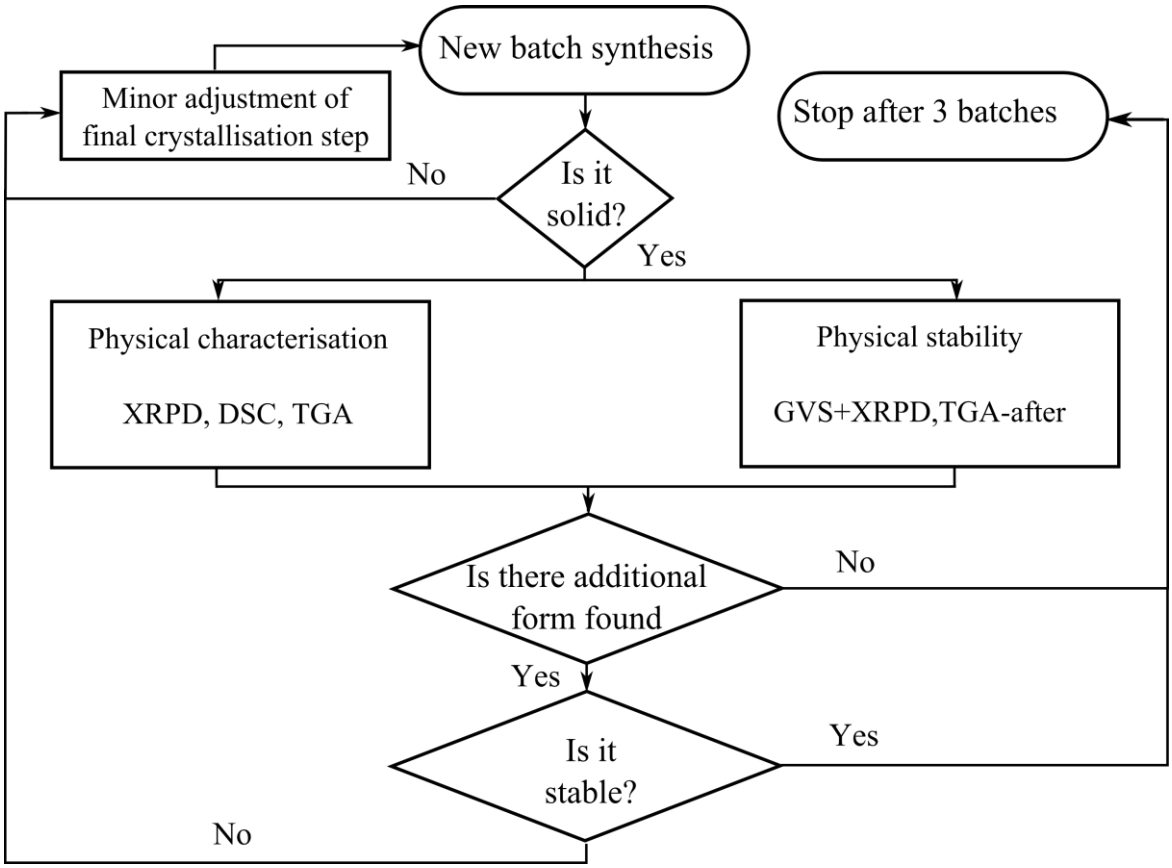



*Figure 11 Flowchart for early polymorphism and solvatomorphism tendency and stability evaluation of a new drug candidate.(Modified from Publication I).*

### 5.1.2 Solid-state forms of the new drug candidate and relative stability of the discovered forms, I

On the basis of the PXRD measurements and complementary analysis of the batches by TGA, DSC and GVS (I, Table 1, Figs. 3, 6 and 7), the following three crystalline solid forms of ORM10921·HCl were identified: (i) anhydrate I (AHI), (ii) anhydrate II (AHII), and (iii) hemihydrate I (HH).

The two anhydrous forms, AHI and AHII, have been obtained by crystallization from a dry solvent, applying all possible precautions to avoid any contact of the sample with atmospheric moisture. The anhydrous nature of the forms was verified by TGA analysis (I, Table 1). The forms have different melting points and enthalpies (for AHI, $T_{onset}$=199 ºC and $\Delta H_f$ = 65 J/g, and for AHII, $T_{onset}$ = 210 ºC and $\Delta H_f$ = 80 J/g). Moreover, they were found to be readily further differentiated by distinctive profiles of the moisture sorption isotherms (I, Fig. 6). Interestingly, despite the crystalline nature of AHI and AHII, the moisture-induced phase transitions have been observed for both forms. The critical RH for the phase transition was 40-60% for AHI and 93% for AHII. Similar to the case of the predominantly amorphous initial material, the crystalline phase resulting from this phase transformation was identified to be HH in all cases (I, Table1).

A common tendency of all amorphous and anhydrous forms to undergo solid-phase transformation into HH suggests that HH has a higher physical stability. This makes HH attractive in view of pharmaceutical applications. The formation of the desired HH form was achieved from a mixture of ethyl acetate and water. The GVS analysis of batches B6-B8 has proven that HH is indeed a stable crystalline material (I, Fig.6).

In order to facilitate the process of the solid form selection for the early drug development, the stability relationships between the four discovered solid forms of ORM10921·HCl — amorphous form (AM), anhydrate I (AHI), anhydrate II (AHII), and hemihydrate (HH) — has been deducted on the base of the study. Overall, with respect to physical stability, these solid-state forms can be ordered at ambient conditions as follows: HH > AHII > AHI > AM. In fact, due to the superior relative stability of HH, the other three forms exhibited a tendency to convert into HH at high relative humidity, as schematically illustrated in Figure 12. Furthermore, comparison of thermal properties of the two anhydrous forms revealed higher melting temperature and melting enthalpy of the form AHII. In accordance with the Burger-Ramberger rule, this is an indication of monotropically related polymorphs, with the form AHII being the most stable anhydrous form at all temperatures.

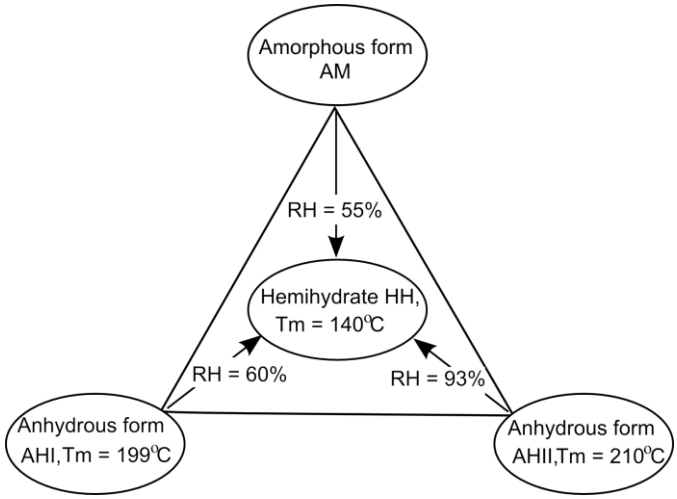



*Figure 12 Schematic representation of the relative stability between the discovered solid forms of ORM10921·HCl. (Modified from Publication I).*

### 5.1.3 Insights into relationship between the structure and the properties, I

The single-crystal X-ray diffractometry showed that, in the HH crystal (I, Fig. 5B), water molecules play the role of binding agents, connecting two different host molecules via the H-bond with $Cl^-$. To gain insight into the hydrate formation by ORM10921·HCl, the Cambridge Structure Database (CSD) search was performed for hydrates of HCl salts of organic molecules that contain a tertiary N atom and only ether's O atoms as H-bond acceptors. This research revealed that, in all such molecules, $Cl^-$ is the only structural element participating in the formation of H-bonds with water. Furthermore, $Cl^-$ was found

to be able to accept as many as six H-bonds. This ability of $Cl^-$ is exemplified, for instance, by the crystal structure of VORMEU (CSD reference code). In addition, water molecules are prone to form their own hydrogen bonding networks. Taking into account these factors, one could expect to find also the hydrates with higher numbers of water molecules. However, only the lowest hydrate form, the hemihydrate (HH), was discovered in this study. In conclusion, the above structural considerations suggest that a comprehensive polymorph and solvate screenings should be performed at the later development phases of the project in order to discover all possible solid-state forms of ORM10921·HCl.

## 5.2 Salt-cocrystal continuum for itraconazole, II-IV

Itraconazole (ITZ) is an antifungal drug invented by Janssen Pharmaceutica in 1984. The commercial name of this drug is Sporanox. The physical and chemical properties of ITZ are similar to many other important drug candidates: high lipophilicity log*P* = 6.2, very weak basicity pKa = 3.7, high molecular weight Mv = 705.6 g/mol, lack of H-bond donors in the structure, and low solubility in aqueous media. This makes ITZ an excellent model molecule for diverse pharmaceutical studies.

### 5.2.1 Reinspection of the weak bonding interactions in itraconazole: succinic acid cocrystal and neat itraconazole, II

In 2003 Remenar *et al.* have obtained ITZ cocrystals with 1,4 - dicarboxylic acids that exhibited significantly enhanced water solubility compared to that of the parent drug. In addition, a supramolecular trimer of the ITZ-succinic acid 2 (Figure 13) has been discovered. The trimer was considered as a valuable example of a molecular fitting mechanism in crystal engineering. It is remarkable that the interaction between succinic acid and the strongest basic group of ITZ (piperazine moiety) was not present in the co-crystal structure (Remenar *et al.* 2003). Instead, the COOH-group of succinic acid was found to be connected to a 1,2,4-triazole group, which was not an expected result.

During the course of our investigations of the cocrystal of ITZ and succinic acid it has been noticed that the modeled cocrystal structure (CSD:12 IKEQEU) has its carbon, hydrogen, and nitrogen atoms wrongly placed in the 1,2,4-triazol-5-one ring. In addition, we noted that the structure (CSD: TEHZIP) of the neat ITZ has been determined at room temperature (RT) with a quite limited resolution. These aspects prompted us to undertake additional investigations of the cocrystal and the neat ITZ and reaffirm the existence of the trimer in the cocrystal.

To correct chemical composition of the triazolone ring in the cocrystal, the relevant chemical elements have been properly defined in our new structure model. Then, an enhanced disorder model has been proposed by us for both the compounds along with a proper interpretation of the corresponding intermolecular interactions. In this model we

suggested a tilt of triazolone ring (highlighted by yellow in Fig. 13) in addition to a disorder in the sec-butyl groups presented in conventional models.

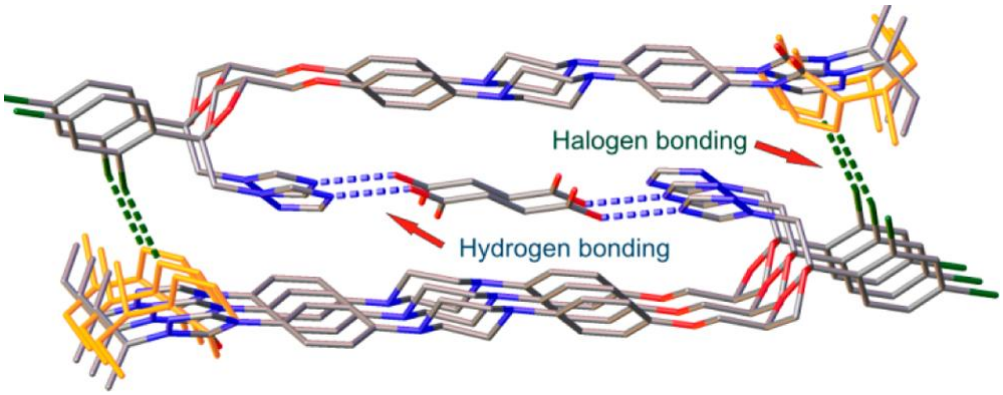



*Figure 13* *Supramolecular trimer unit formed by H-bonding between the-COOH moieties of succinic acid and 1,2,4-triazole groups of itraconazole and the discovered halogen bonds between the tilted triazolone and dichlorophenyl groups of itraconazole*

Our results confirmed that the neat ITZ crystallizes in a triclinic crystal system that shows two conformationally distinct molecules A and B in the asymmetric unit (II, Figure 2b). The main difference between the conformations lies in their methoxyphenyl-piperazine moiety that is tilted in the B conformation by about 90°, in contrast to the phenyl ring adjacent to a piperazine. It can also be noted that the geometries of various homo- and heterocyclic rings in the structure are consistent with the ring geometry reported earlier (folded dioxolane and chair-like piperazine). However, for the neat ITZ, new interesting structural features associated with the intermolecular interactions in the crystal lattice were found. As an example, we have found weak halogen bonding contacts (Cl1c···O17c = 3.01 Å and 3.08 Å at -150 and 0 °C, respectively) that take place between dichlorophenyl and dioxolane groups in a dimeric arrangement (II, Figure 3a). These contacts are revealed only between two adjacent molecules of the B conformation, whereas in a combination A-A, the contact distances remain outside the boundaries of halogen bond criterion, being about 3.8 Å. In addition to the halogen bonding contacts, only a weak H-bonding interaction C–H···N exists between triazole and triazolone rings. Furthermore, there is a weak face-to-edge π–π stacking network formed via phenyl and methoxyphenyl groups of neighboring molecules. The π–π stacking is enabled by the ~90° tilt of the methoxyphenylpiperazine moiety of the B conformation.

Our results also confirmed that the ITZ–succinic acid cocrystal underwent crystallization in a monoclinic crystal system that shows a single ITZ molecule with a half of the succinic acid molecule in the asymmetric unit. Thus, the formation of the supramolecular trimer, in which succinic acid is hydrogen-bonded between two ITZ molecules (II, Figures 2 and 3), was verified. Moreover, the lack of the H-bonding interaction between the most basic group of ITZ (piperazine) and succinic acid was

confirmed. On the other hand, in the original structure, the misplaced C–H formed an exceptionally short artificial contact with a neighboring sec-butyl group (the mean H···H distance 1.821 Å). In our structure, with a proper determination of the positions of the C–H and N atoms and with an enhanced disorder model, the halogen bonding contacts (C–Cl···N (Cl1···N41b) 2.836(15) Å and 2.97(2) Å at -150 and 0 °C, respectively) were discovered between the disordered triazolone ring and the dichlorophenyl group of the neighboring ITZ molecule (II, Figure 3b), and no artificial short contacts have been found. Therefore the observed halogen bonding in conjunction with the stronger H-bonding with succinic acid is responsible for the packing of the trimers in the solid-state self-assembly of the cocrystal. The π-stacking occurring in the neat ITZ is not present in the cocrystal, as trimeric units are packed in rows along the **b** axis and these rows are parallel to the **a** axis.

### 5.2.2 Preliminary molecular modeling, III

Complementarities between functional groups and a host-guest geometric fit are the key prerequisites for a successful cocrystal formation (Aakeröy *et al.* 2012). As we have confirmed in the Publication II, in the supramolecular trimer of ITZ-SUC the guest molecule of succinic acid is located in a pocket formed by two ITZ molecules and H-bonded with the 1,2,4-triazole groups, serving as a bridge between the two host molecules (Fig. 14). We assumed that similar supramolecular motifs can exist in other cocrystals of ITZ with C4 dicarboxilic acids. In our structural considerations we have assessed the possibility to obtain analogous supramolecular trimers between ITZ and other dicarboxylic acids by systematically replacing the SUC with dicarboxylic acids of varying carbon chain lengths. Initially, the succinic acid was replaced with one of the dicarboxylic acids and then the ITZ molecules were added to the acid so that the H-bonding and ITZ geometry stays the same as in the ITZ-SUC cocrystal. The conformations of dicarboxylic acids (III, Figure 3 b-e) used in this study were selected in a way that ensures the pocket geometry of ITZ-SUC in the model structures. Here we exploited a mix and match strategy described in (Aakeröy *et al.* 2012). Based on these structural considerations, we have concluded that the geometries similar to that of ITZ-SUC trimer can be realized with a variety of dicarboxylic acids as guest molecules. It should be noted, however, that the formation of such trimers with the odd-chain dicarboxylic acids requires a significant departure from their minimal energy conformation, as the latter is characterized by an angle of approximately 120° between the two –OH groups (Aakeröy *et al.* 2012; Gopalan et. al. 2000). In contrast, the even-chain dicarboxylic acids are known to have the minimum energy conformation with the –OH groups arranged in a collinear fashion (Gopalan et. al. 2000; Aakeröy *et al.* 2012). As a result, the replacement by a dicarboxylic acid with an even number of carbon atoms allowed a trimer formation using the conformation of the acid with minimum energy. This suggests a higher probability of the cocrystal formation with these acids.

In the previous subsection we have shown that the revised structures revealed the presence of weak halogen bonding as one of the important non-covalent interactions in ITZ-SUC cocrystal (N…Cl) (Fig.13) as well as in neat itraconazole (O…Cl).

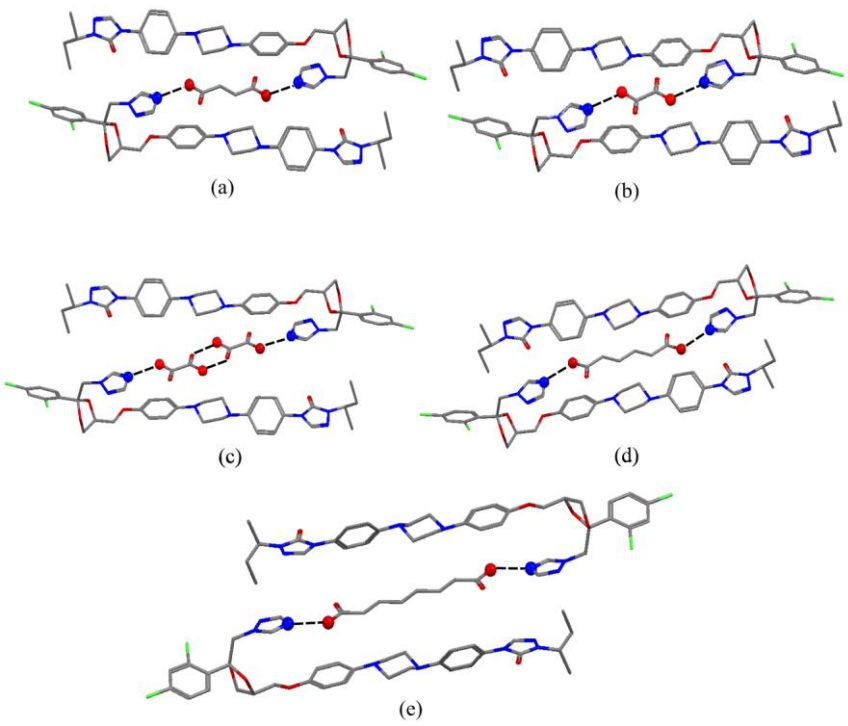



*Figure 14 The examples of hypothetic trimers that can be formed between itraconazole and dicarboxylic acids with varying carbon chain length.*

Our modeling suggested that the length alteration of the carbon chain of acids forming the trimer leads to a shift of the relative position of the itraconazole molecules in opposite directions. This shift changes the outer size and the geometry of the supramolecular trimer and, as a result, may significantly alter the weak halogen-bonding network between the trimers in the ITZ cocrystals. Since weak van der Waals interactions and halogen bonds both participate in bonding and stabilizing the trimers in the cocrystal, it is difficult, if not impossible, to predict whether or not these virtual trimers would eventually form a periodic crystalline structure.

### 5.2.4 Obtaining cocrystals and salts of itraconazole, III-IV

#### *Screening for cocrystals of itraconazole and dicarboxylic acids, III*

According to the structural considerations, ITZ should be able to form cocrystals with dicarboxylic acids that have an even number of carbon atoms in the chain. To verify this hypothesis we have performed an experimental screening that employs dicarboxylic acids of varying chain length (C2-C10), including also those that have an odd number of carbon atoms. The experimental protocol and decision tree used in our cocrystal screening.

approach is introduced in the Fig.15. In order to maximize the number of possible hits, we used both mechanochemical synthesis by solvent assisted grinding and slow evaporation techniques (Sheikh *et al.* 2009) and we made both 1:1 and 2:1 (ITZ: coformer) mixtures of the components. The physical and chemical analyses of the prepared substances were performed by using the same methodology focused on disclosing and thorough investigation of the nature of new crystalline compounds.

The success and advantage of the solvent drop-grinding technique in synthesis of cocrystals, as discussed, e.g., in (Chiarella *et al.* 2007), is mainly due to the low amount of solvent. This observation dictates that the crystallization takes place in a region of the phase diagram in which the co-crystal is more likely to be a stable solid phase. The previously reported unsuccessful attempts to obtain the cocrystal of ITZ and MAL were based on high throughput crystallization from solution that at least requires the ternary phase diagram to be taken into account (Chadwick *et al.* 2009). Thus, in our slow evaporation crystallization experiments we used the mixture of CLF and THF that allows achieving of the congruent crystallization due to comparable solubility of both components in this solvent mixture.

In the experiments, we have indeed obtained cocrystals of ITZ with oxalic (C2) and adipic (C6) dicarboxylic acids, of which two are cocrystal solvates (ITZ-OXA-ACE and ITZ-OXA-THF) and one a hydrate (ITZ-ADI-H2O). Then, to our surprise, we have succeeded in obtaining the cocrystals of ITZ with the malonic (C3), glutaric (C5), and pimelic (C7) acids, which have an odd number of carbon atoms in the chain. These cocrystals are ITZ-MAL, ITZ-GLU, and ITZ-PIM. However, we could not obtain cocrystals of ITZ with the C8-C10 dicarboxylic acids. Also, the solvent assisted milling of ITZ with azelaic (C9) acid resulted in a mixture of pure ITZ and alpha polymorphic form (Form II) of azelaic acid reported in (Housty 1967).

In terms of cocrystal generation, both screening methods - solvent assisted milling and slow evaporation – were equally successful. The main difference between the methods is that they yielded different cocrystal solvates, which is solely attributed to the different solvents employed during the experiments, as in the case of ITZ cocrystals with oxalic acid. In addition, the slow evaporation experiments were able to produce ITZ-ADI-H2O, while the solvent assisted milling resulted in a physical mixture of ITZ and ADI only.

On the other hand, the solvent assisted milling experiments of ITZ and the GLU acid in 1:1 and 2:1 ratios indicated the existence of a ITZ-GLU cocrystal of a different stoichiometry (1:2), which was not achieved in the crystallization experiments.

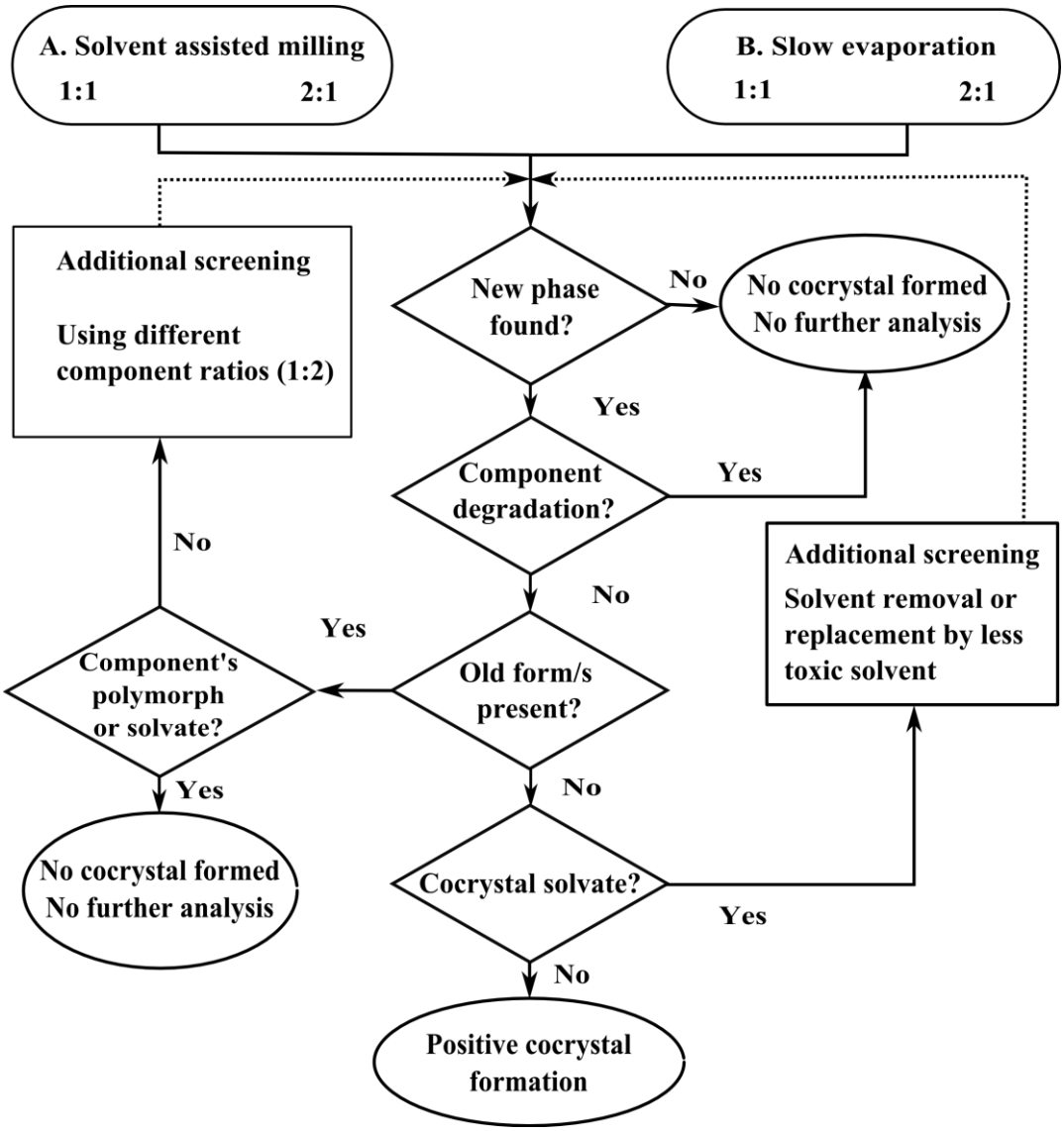



*Figure 15 The decision tree used for screening experiments.*

The detail description of the properties of the new cocrystals can be found in Publication IV and in the upcoming section 5.3.

The results demonstrate that employing the combined screening approach, where the two most popular experimental techniques (Sheikh *et al.* 2009) and various ratios of the components are used, is feasible in order to maximize the number of hits. The initial screening performed by (Remenar *et al.* 2003) used only high-throughput screenings that were based on evaporative crystallization, in which the choice of solvents and the knowledge of the phase diagram are vital for a success. Using this combined approach we have obtained seven multiple component compounds (Table 3). Six of them have not been reported in the scientific literature before.

*Hydrochloride salts of itraconazole, IV*

In order to enable comparison of the properties of the ITZ cocrystals with ITZ salts we have prepared ITZ hydrochloride salts using two traditional approaches described in the section 4.2.2 by gas bubbling and reaction crystallization methods. Unexpectedly, we have obtained two different salt forms. First, the ITZ dihydrochloride (denoted ITZ·2HCl, stoichiometry verified by elemental analysis) showed unique PXRD pattern different from the known ITZ dihydrochloride. Secondly, the new ITZ trihydrochloride (denoted ITZ·3HCl, stoichiometry verified by elemental analysis) was obtained in the gas bubbling experiments. The detailed description of the properties of the salts can be found in Publication IV and in the upcoming section 5.3.

### 5.2.5 Solid-state properties of salts and cocrystals of itraconazole, III-IV

Table 3 summarizes the solid state properties of the multiple component compounds discovered in our experiments. The unique PXRD patterns of the salts, cocrystals and ternary compounds are shown in (III, Fig. 5 and IV, Fig. 3a).

More specifically, two new cocrystals of ITZ and C3 and C5 dicarboxylic acids were obtained as a pure phase in both grinding and slow evaporation experiments. First, ITZ and malonic acid (C3), denoted ITZ-MAL, 2:1, had the same stoichiometry, determined by NMR, as the known cocrystal of ITZ and succinic acid (ITZ-SUC, 2:1). Second, the cocrystal of ITZ and glutaric acid C5 (ITZ-GLU), surprisingly, has substantially different stoichiometry 1:2. The pure ITZ-GLU was obtained from additional experimental cycle using 1:2 molar ratio of ITZ to acid by both evaporation and grinding experiments. All three cocrystals had different melting points. Interestingly, the melting points of the ITZ-MAL and ITZ-SUC lies between the melting points of neat ITZ and the coformer. However, the melting point of the ITZ-GLU is lower then the melting points of both components (table 3). In addition, the empirical evidence of existence of the ITZ and pimelic acid C7 cocrystal (ITZ-PIM) has been demonstrated. However, the stoichiometry and physical properties of the ITZ-PIM were not possible to study, since the form has not been obtained as a pure phase.

Furthermore, three new ternary component compounds were obtained. Two solvates of ITZ and oxalic acid C2 cocrystals with acetone (obtained by solvent assisted milling) and tetrahydrofurane (obtained by slow evaporation) denoted ITZ-OXA-ACE (1:1:0.5) and ITZ-OXA-THF (1:1:1), respectively. Last, the hydrate of ITZ and adipic acid C6 cocrystal denoted ITZ-ADI-$H_2O$ (1:1:1.3) was obtained in slow evaporation experiments. The stoichiometry and solvation nature of the materials were verified by TGA coupled with MS analysis. The solvent displacement by drying or moisturizing failed, leading to collapse of the crystalline structure for all solvates.

No cocrystals were formed between the ITZ and C8-C10 acids, which are suberic, azelaic and sebacic acids. In the publication III, subsections 3.3.1-3.3.3, the results are described in more details individually for each drug-coformer combination. It must be noted that NMR and HPLC showed no signs of degradation in all samples analyzed.

**Table 3.** *Solid state properties of salts and cocrystals of itraconazole (Modified from III and IV).*

| Cocrystal/ Cocrystal solvate | Number of carbons | Stoichio-metry | Co-former Mp, °C | Co-crystal Mp (Desolvation/ Desproportionation T ) °C | Weight loss on drying, % | De-solvation product |
|---|---|---|---|---|---|---|
| ITZ-MAL (III, IV) | 3 | 2:1 | 134 | 148 | 0 | n.a. |
| ITZ-SUC (III,IV) | 4 | 2:1 | 186 | 157 | 0 | n.a. |
| ITZ-GLU (III) | 5 | 1:2 | 98 | 94 | 0 | n.a. |
| ITZ-PIM (III) | 7 | n.a. | 104 | n.a. | 0 | n.a. |
| ITZ-ADI-$H_2O$ (III) | 6 | 1:1:1.3 | 153 | (80) | 2.8 | Am |
| ITZ-OXA-ACE(III) | 2 | 1:1:0.5 | 189 | (81) | 3.4 | Am |
| ITZ-OXA-THF (III) | 2 | 1:1:1 | 189 | (100) | 7 | Am |
| ITZ·2HCl (IV) | - | 1:2 | -114.9 | $(90)^2$ | 10.8 | n.a. |
| ITZ·3HCl (IV) | - | 1:3 | -114.9 | $(90)^2$ | 11.2 | n.a. |

The hydrochlorides ITZ·2HCl and ITZ·3HCl were white crystalline powders with unique PXRD patterns (IV, Fig 3a) different from the ITZ dihydrochloride reported in literature (Remenar *et al.* 2003; Tao *et al.* 2009). None of the salts, however, exhibited any DSC endotherm attributable to the melting. Instead, the slow loss of weight started as low as at T > 40 ºC with a considerable rate increase at T > 90 ºC and between 90 and 250 ºC equals 10.8% and 11.2% for ITZ·2HCl and ITZ·3HCl. The increase in the weight loss rate corresponds to the first broad endotherm observed in the DSC thermograms for the salts (IV, Fig. 3b). Moreover, the MS analysis performed simultaneously with TGA indicated the evaporation of only Cl ion that correlates with the weight loss kinetic giving the firm evidence of disproportionation of the salt. The revealed thermal behavior of ITZ hydrochloric salts is similar to that of the ITZ dihydrochloride described previously (Tao *et al.* 2009).

Such variety of ITZ hydrochlorides is likely due to its very weakly basic properties as well as molecular structure with multiple proton accepting sites that probably leads to

variability of HCl location in the solid state. This fact makes it challenging to establish a robust process of crystallization of a certain form.

### 5.2.6 Molecular insight into formation of itraconazole multicomponent compounds with dicarboxylic acids, III

In order to gain insight into the obtained new non-solvated crystalline forms, an experimental analysis combining 13C cross-polarization magic-angle spinning (CPMAS), solid-state NMR (SSNMR), and Raman spectroscopy was performed (Elbagerma *et al.* 2010; Vogt 2010). In this study, 13C CPMAS NMR and Raman spectra of the cocrystals were compared with those of the pure ITZ and dicarboxylic acids.

In solid forms, most carboxylic acids appear as H-bonded chains in all polymorphic forms, where the carboxyl groups of one molecule are H-bonded to the carboxyl groups of the neighboring molecules, so that C=O groups act as acceptors and OH groups as donors (Gopalan *et al.* 2000). In the known ITZ-SUC cocrystal, the supramolecular trimer consists of two ITZ molecules that are H-bonded to succinic acid in such a way that the C=O group of the acid is free of H-bonds, but the –OH group is connected to the 1,2,4,-triazol group of ITZ (Fig. 13). This implies that, in the Raman spectrum of the cocrystal, the C=O stretching vibration must be shifted to a higher wavenumber (Socrates 2004). Indeed, the C=O peak observed in the Raman spectra of SUC at1653 cm-1 is shifted to 1719 cm-1 in the ITZ-SUC spectrum (III, Fig.6). Moreover, in the SSNMR, the 13C signal from carbonyl (C=O) in succinic acid (180.0 ppm) showed an upfield shift (175.0 ppm) of 5.0 ppm (III, Fig. 7). The neat malonic acid displayed a doublet resonance pattern (C=O at 174.7, 174.3 ppm) due to the presence of two non-equivalent H-bonding schemes in the crystal: an intramolecular and an intermolecular35. Similarly, in the Raman spectra of the neat malonic acid, two peaks attributed to the C=O vibrations are seen at 1650 $cm^{-1}$ and 1680 $cm^{-1}$ (III, Fig. 6). Interestingly, the ITZ-MAL cocrystal resulted in a singlet resonance pattern with the carbonyl signal showed an upfield shift (169.0 ppm) of 5.7 ppm. Also, in the Raman spectra of ITZ-MAL, there is a shift of the C=O vibration to a higher wavenumber (1730 $cm^{-1}$). In addition, the neat ITZ shows a doublet resonance pattern in its solid state NMR attributed to the presence of two non-equivalent molecules in an asymmetric unit of the crystal lattice (III, Nonappa *et al.* 2013). The cocrystal with succinic acid (ITZ:SUC) shows a significant change in the SSNMR spectra exhibiting a singlet resonance pattern that indicates the presence of one molecule of ITZ in an asymmetric unit. These results are consistent with the single crystal X-ray studies (III, Nonappa *et al.* 2013). The cocrystals obtained with malonic acid also showed a similar change in the SSNMR. Overall, the 13C CPMAS spectra of ITZ-MAL are very similar to the ITZ-SUC. This clearly illustrates a similarity of the ITZ:SUC and ITZ:MAL cocrystal structures, which is a remarkable result.

Furthermore, in Raman and CPMAS NMR spectra of all cocrystals and cocrystal solvates discovered in this work (III, Figs. 6 and 7), we have noticed the shifts of the C=O vibration peak to a higher wavenumber (in Raman spectra) and upfield shifts of the 13C signal from the carbonyl (C=O) of the carboxylic acids (in CPMAS NMR spectra). This is

an indication of the absence of the H-bonding between the C=O and OH groups due to formation of a complex with ITZ. However, the other cocrystals and cocrystal solvates has the SSNMR spectra that are distinctive from the ITZ-SUC and ITZ-MAL due to a different stoichiometry.

Most importantly, C7 was identified as the maximum carbon atom number of the aliphatic chain for the successful cocrystallization reaction between ITZ and a dicarboxylic acid. In terms of supramolecular structures, two families of the ITZ cocrystals were recognized: (1) the cocrystals built of the trimer characteristic of ITZ-SUC, and (2) the cocrystals that are not able to form similar synthons. These results demonstrate that a preliminary molecular modeling, with only one conformation of ITZ being considered (the one that is realized in ITZ-SUC), is not enough for a reliable prediction of obtainable cocrystals of ITZ and aliphatic dicarboxylic acids, as these molecules are rather complicated and conformationally flexible. Such modeling, however, might still help to find the direction of searching for probable successful hits.

## 5.3 Pharmaceutical properties of cocrystals and salts of itraconazole, IV

This subsection deals with the properties of the neat ITZ, two cocrystals ITZ-MAL and ITZ-SUC and two new hydrochloric salts ITZ·2HCl and ITZ·3HCl with main focus put on the hygroscopicity, stability (including disproportionation) and intrinsic dissolution.

### 5.3.1 Hygroscopicity and relative thermodynamic stability at ambient conditions, IV

In order to evaluate the hygroscopicity and compare the thermodynamic stabilities of the cocrystals and salts at ambient conditions the GVS method was used. Commercial ITZ, being a hydrophobic, non-porous, and crystalline material, shows very low hygroscopicity (IV, Fig. 4a). The two cocrystals, ITZ-SUC and new ITZ-MAL, exhibited a quite similar non-hygroscopic behavior comparable with that of the commercial ITZ (IV Fig. 4a). The GVS sorption-desorption isotherms were reversible, showing almost no hysteresis.

In contrast, both ITZ hydrochloric salts are highly hygroscopic at RH > 70 % (IV, Fig 4b). No deliquescence was observed with ITZ·2HCl under the conditions of the experiments. The ITZ·3HCl exhibited higher moisture uptake at RH > 75 %. Moreover the deliquescence occurred, and the sample transformed into a highly viscose transparent substance. This change was irreversible and the sample mass decreased by 3 % after the desorption indicating an instability of the salt.

### 5.3.2 Solubility and intrinsic dissolution rate (IDR), IV

The apparent solubility of all substances at T = 37 ºC in the dissolution medium was determined by the shake–flask method before the IDR method had been developed (table 4). A 10-mg sample equilibrated for 3 days with 20 ml of the buffer.

The intrinsic dissolution profiles within the first 30 min of the studied systems are shown in publication IV (Fig. 5). The calculated IDR are presented in Table 4. The highest

**Table 4.** *Properties of itraconazole solid forms studied.(Modified from IV)*

| Solid form | Melting temperature | Disporoportionation (Decomposition) temperature | IDR | Solubility | Relative kinetic stability |
|---|---|---|---|---|---|
| | ºC | ºC | pH 1.2, µg $min^{-1}$ $m^{-2}$ | µg/mL | grades 10-0 |
| ITZ | 169 | n.o. | 0.95 | 5 | 10 |
| ITZ·2HCl | 90 | 90 | 45.9 | 26 | 5 |
| ITZ·3HCl | 90 | 90 | 35.4 | 74 | 3 |
| ITZ-SUC | 157 | n.o. | 11.0 | 18 | 10 |
| ITZ-MAL | 148 | 148 | 4.5 | 17 | 10 |

IDR was observed for the both hydrochloric salts (ITZ·2HCl and ITZ·3HCl). The superior IDR and solubility demonstrated by hydrochloride is obviously due to ITZ being protonated (subsection 5.2.5 and IV, SI). The dissolution rate of ITZ-SUC cocrystal was ~11-folds higher than the dissolution rate of the commercial ITZ. Finally, the new ITZ-MAL cocrystal exhibited ~5-fold higher IDR in relation to ITZ.

### 5.3.3 Water induced transitions and stability of itraconazole salts and cocrystals, IV

To reveal possible solid-phase transitions induced by moisture, the GVS and IDR tests were combined with PXRD analysis performed before and after the experiments. Comparison of PXRD patterns before and after GVS and IDR revealed no structural changes in the ITZ-MAL and ITZ-SUC cocrystals as well as in commercial crystalline ITZ. However, the PXRD pattern measured after the GVS showed ITZ·2HCl to transform into another solid form (IV, Fig. 6a). Similarly, the PXRD pattern of ITZ·2HCl, acquired after the IDR experiments, proves that the salt undergoes a phase transformation during the dissolution. The resulting solid form is identical to the one found after the GVS

experiments, and is presumably a hydrate of the ITZ hydrochloric salt. Furthermore, after 30-60 min after the dissolution test was initiated, gas bubbling was noticed on the surface of disc of the ITZ·3HCl samples. The PXRD analysis performed after the IDR experiments indicates a complete amorphization of the ITZ·3HCl sample (IV, Fig. 6b). We presume that this phenomenon is caused by the HCl gas liberation from the samples during the IDR experiments. This phenomenon once again refers to instability of ITZ·3HCl under the experimental conditions. Moreover, the same amorphization took place in the GVS experiment (IV, Fig. 6b), which proves that the effect is triggered upon the contact with water molecules. Since we have noticed the disproportionation of both salt forms upon heating (Table 3 and 4) the possible explanation for such phenomenon could be a disproportionation of the salt (Stephenson and Taylor 2011).

Overall, based on the obtained results, it is possible to rank the studied forms in terms of relative thermodynamic stability at ambient conditions as follows (grades 10-0): 10 ITZ ≈ 10 ITZ-SUC ≈ 10 ITZ-MAL > 5 ITZ·2HCl > 3 ITZ·3HCl. Interestingly, the same ranking between the studied forms is possible to establish on the basis of the DSC results. The ITZ having a highest melting point ($T_{m,onset}$ = 169 °C) and zero weight loss is leading, followed by cocrystals ITZ-SUC ($T_{m,onset}$ = 157 °C) and ITZ-MAL ($T_{m,onset}$ = 148 °C). These solid forms exhibit a very high thermal stability and are stable also at high relative humidity. Therefore, they can be used in formulation without any special stabilizing. In the contrast, the two hydrochlorides of ITZ are not stable upon heating and decompose at T > 90 ºC. Such instability of the ITZ hydrochloric salts can lead to numerous difficulties in establishing a robust formulation based on them.

Among the solid-state forms reported in this work, the cocrystals ITZ-SUC and ITZ-MAL are most promising in view of pharmaceutical applications, since they do enhance the dissolution rate of ITZ and yet exhibit superior stability. The study demonstrates that for any weak bases, cocrystallization is a more suitable approach, as compared with hydrochloric salt formation.

# 6 Summary and conclusions

(1) Fast and cost-effective assessment of polymorphism and solvatomorphism tendency and physical stability of the new drug candidate ORM10921 hydrochloride has been accomplished. Three new crystalline forms, including two anhydrous and one hemihydrate, have been discovered. The relative stability of the discovered solid forms at ambient conditions was established, and the most stable form was found to be the hemihydrate. The single-crystal structure revealed the Cl- atom to be responsible for hemihydrate formation. The procedure can be recommended for the evaluation of the polymorphism and solvatomorphism tendency of hydrochlorides salts of new chemical entities ("precandidates") even prior to the final selection of the drug candidate.

(2) In order to enable molecular modeling simulations the more accurate crystalline structures of both neat itraconazole (ITZ) and itraconazole-succinic acid (ITZ-SUC) cocrystal were revealed. A weak halogen bonding in the crystals has been found. The molecular modeling simulations have been performed. The experimental results demonstrate that a preliminary molecular modeling, with only one conformation of ITZ being considered (the one that is realized in ITZ-SUC), is not enough for a reliable prediction of obtainable cocrystals of ITZ and aliphatic dicarboxylic acids, as these molecules are rather complicated and conformationally flexible. Such modeling, however, still helps to find the direction of searching for probable successful hits. Together with the screening technology, this approach can considerably improve the cost efficacy and probability of the success.

(3) The optimal salt and cocrystal screening approach based on mechanochemistry synthesis and slow evaporation was used to reveal 9 new multicomponent crystalline solid forms. We have systematically studied cocrystallization of ITZ with a range of aliphatic dicarboxylic acids. Most importantly, C7 was identified as the maximum carbon atom number of the aliphatic chain for the successful cocrystallization reaction between ITZ and a dicarboxylic acid. In terms of supramolecular structures, two families of the ITZ cocrystals were recognized: (1) the cocrystals built of the trimer characteristic of ITZ-SUC and (2) the cocrystals that are not able to form similar synthons. Overall, our work demonstrates a wide diversity of ITZ cocrystals with aliphatic dicarboxylic acids having a variety of the carbon chain lengths. This finding has a considerable conceptual and practical value in the field of crystal engineering, putting an additional emphasis on the importance of the weak intermolecular interactions in the crystal structure cohesion.

(4) The comparative study of the pharmaceutical properties of the new ITZ salts di- and tri-hydrochlorides and new ITZ-malonic acid (2:1) cocrystal has been performed. The intrinsic dissolution rate, hygroscopicity, and thermodynamic stability were determined for the obtained solid-state forms and compared to those of the ITZ-SUC (2:1) cocrystal. The results showed that the forms with higher dissolution rates are less stable. The reported research demonstrates that, for weak bases, cocrystallization is a more suitable approach to the dissolution rate enhancement of poorly water-soluble drugs, as compared with the conventional hydrochloric salt formation.

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