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ENANTIOMERS AND POLYMORPHISM IN VENLAFAXINE AND ONDANSETRON

een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

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Chapter 1
INTRODUCTION

1.1 Polymorphism in molecular crystals

The term polymorphism is used in many different areas. Threllfall\(^1\) found 1.5 million hits in an internet search, 90% of them referred to video games, of the remainder, 90% referred to genetic polymorphism, which involves minor changes in protein and/or DNA sequences. Of references dealing with crystallographic polymorphism only 10% deals with molecular crystals, the subject of interest of this thesis. McCrone defines a polymorph as ‘a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state’\(^2\). The amorphous state is sometimes also regarded as a polymorph. However, because amorphous forms are not crystalline, they consist of a disordered arrangement of molecules, and are not a true polymorph. In the area of crystallography Mitscherlich is regarded as the first to use the term polymorphism\(^3\), although in fact other researchers reported earlier more than one crystal form for a chemical compound; Klaproth described the fact that two different crystals, aragonite and calcite had the same chemical composition\(^4\), see figure 1, and Davey found that diamond was a form of carbon\(^5\), see figure 2. Figure 3 shows two different forms of sulphur, the monoclinic and the orthorhombic form. Polymorphism of elements is called allotropy. For a more elaborate overview of the history of the development of polymorphism see Bernstein\(^6\) and references therein.

Figure 1: Two polymorphs of CaCO\(_3\), left calcite and right aragonite
McCrone’s definition of polymorphism seems straightforward. It includes conformational polymorphism, in which flexible molecules can adopt different conformations in the different crystal structures, but dynamic isomerism and tautomerism are excluded because in these cases different molecules are formed. A safe criterion to classify a system as polymorphic would be: different crystal structures that form the same liquid and vapour states. A system for which the isomers (or conformers) can interconvert rapidly in solution, melt or vapour are considered
polymorphic according to this criterion. Dunitz stated that this definition in case of a chiral compound, a racemate and a conglomerate are classified as polymorphs, if the interconversion of the enantiomers in the melt or in solution is fast but as three different compounds in case the interconversion is slow. A term that is related to polymorphism is pseudopolymorphism. The term is used to describe solvates of a compound, for which the solvent is part of the crystal structure. In case of water as solvent we speak of a hydrate. Despite the fact that the term pseudopolymorphism is widely accepted, especially in the pharmaceutical industry, it is better changed to solvate. In any case a solvate and an unsolvated crystalline form are constitutionally distinct and can therefore not be defined as polymorphs.

Two polymorphs of a compound have different unit cells. As a result of this different polymorphs can exhibit a variety of different physical properties; table 1 lists some of the properties that differ among different polymorphs.

<table>
<thead>
<tr>
<th>Table 1. List of some physical properties that can differ among different polymorphs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar volume and density</td>
</tr>
<tr>
<td>Hygroscopicity</td>
</tr>
<tr>
<td>Melting temperature</td>
</tr>
<tr>
<td>Heat capacity</td>
</tr>
<tr>
<td>Solubility</td>
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<tr>
<td>Dissolution rate</td>
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<td>Stability</td>
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<td>Habit</td>
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<td>Hardness</td>
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<td>Compactibility</td>
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In recent years polymorphism of drugs has been the subject for intense interest of the pharmaceutical industry, because the difference in solubility between polymorphs of a pharmaceutical ingredient can affect the efficacy, the bioavailability and the safety of the drug. A lot of effort is put in finding the most stable polymorph of a pharmaceutical ingredient, although no method can provide absolute confidence that the most stable polymorph has been found. A relatively recent example for which the diversity of the bioavailability of the drug proved to be important is Ritonavir. After having been on the market for almost two years, a new
Chapter 1

thermodynamically more stable polymorph, with a lower solubility and hence a lower bioavailability, was found.

1.1 Thermodynamics

The Gibbs free energy, $G$, of a system is defined by the following equation:

$$ G = H - TS. $$

$H$ represents the enthalpy, $T$ the temperature and $S$ the entropy. The entropy of a pure crystalline substance is zero at 0 K and increases with increasing temperature. The enthalpy also increases with temperature and this temperature dependence can be expressed by $C_p$, the heat capacity at constant pressure. The result of the relatively slow increase of $H$ and $S$ with increasing temperature is a decrease of $G$ with increasing temperature $T$. The value of $G$ at 0K is equal to the value of $H$ at 0 K. A system at constant temperature and pressure tends to reach a minimum Gibb’s free energy. Because the thermodynamic properties of different polymorphs are different it is expected that curves of $G$ for different polymorphs are different. Two different types of polymorphic behaviour can be distinguished. Lehmann introduced the terms enantiotropic and monotropic to categorize polymorphic systems. For an enantiotropic system there exists a transition point, the point were the $G$ curves of two polymorphs cross, at a temperature below the melting point of the lower melting polymorph; figure 1a represents such a dimorphic enantiotropic system. Polymorph 4a is the stable polymorph below the transition point and polymorph 2 is the stable one above the transition point. For a monotropic system such a transition point does not exist. In that case polymorph 1 is the stable polymorph at all temperatures as presented in figure 4b.

Considering these energy versus temperature diagrams, several rules of thumb can be derived which are useful for understanding and predicting the polymorphic behaviour. An endothermic heat of transition, for instance, that is observed for a polymorphic transition on heating indicates that the two polymorphs are very probably enantiotropically related. If the observed transition is exothermic the polymorphs are almost invariably monotonically related.

The heat of fusion rule states that if the heat of fusion of the lower melting polymorph is larger than that of the higher melting polymorph, the two polymorphs are enantiotropically related. Conversely, if the higher melting polymorph has the highest heat of fusion, the two polymorphs are monotonically related. These two rules can easily be derived from the curves in figure 4 and have been proven to be very reliable.
Introduction

1.2 Kinetics

Different polymorphs can be obtained by crystallization or precipitation from the solution, the melt or the gas phase. For the pharmaceutical industry crystallization and precipitation from solution are mostly used to obtain different polymorphs, employing various solvents and cooling regimes. Gay-Lussac observed that during crystallization often an unstable form was obtained first, that later transformed to the stable form\(^1\). Ostwald later explained this observation in terms of thermodynamics, formulating his law of successive reactions, also known as Ostwald’s step rule\(^1\). The rule states, “In all processes, it is not the most stable state with the lowest amount of free energy that is initially formed, but the least stable state lying nearest in free energy to the original state”. Although it is only a rule, it proved to be a good starting point to study the kinetics involved in the crystallization process of polymorphs.

The crystallization process concerns various steps. First, in the nucleation stage tiny crystallites are formed in the supersaturated solution. Secondly, in the crystal growth stage these crystallites grow out to form macroscopic crystals until the solution is no longer supersaturated and equilibrium is reached. Smaller crystals have a slightly higher solubility than larger crystals according to the Gibbs-Thomson equation\(^1\). As a result, larger crystals grow at the expense of the smaller ones, a process which is called Ostwald ripening. Nucleation can be a homogeneous process, for which crystals are spontaneously formed in the supersaturated solution or a

Figure 4. The energy versus temperature diagram of a dimorphic system. In (a) an enantiotropic system, and in (b) a monotropic system is presented. \(G\) is the Gibbs free energy, \(H\) the enthalpy. \(MP1\) is the melting point of polymorph 1, \(MP2\) the melting point of polymorph 2, \(T_{trs}\) is the transition temperature.
heterogeneous process, when crystals are formed on foreign material. Both are stochastic processes which are governed by a competition between a volume term and a surface term in the free energy of the nucleus. See figure 5. These terms are different for different polymorphs.

![Diagram of free energy vs. nucleus size](image)

*Figure 5: The activation energy barrier $G^*$ and the critical nucleus size $r^*$ according to classical nucleation theory. $\gamma$ is the surface free energy of the nucleus.*

The most critical step in the formation of different polymorphs is the nucleation step. Depending on the crystallization conditions, the nucleus of a certain polymorph that is formed first, will lead to the formation of that particular polymorph if it grows out fast enough. The conditions can, however, be such that in the supersaturated solution more than one kind of nucleus are formed at the same time\textsuperscript{17}. This phenomenon is called concomitant polymorphism. If concomitant polymorphism is observed at temperatures that differ from the transition temperature, this always involves a non-equilibrium situation. Under the appropriate thermodynamic conditions a less stable polymorph, which was formed initially can be converted to a more stable one. The conversion rate depends on the activation energy and is often mediated by the solution, melt or vapour phase, but can also take place in the solid state itself. Solid-state transformations often have a high kinetic barrier, such that the metastable polymorph can be kinetically stable for very long times\textsuperscript{18}. 

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1.3 Disappearing polymorphs

Several well-documented examples are known in the chemical and pharmaceutical industry of crystal forms that were replaced by new, apparently more stable, polymorphs. Benzocaine picrate is an example; in first instance a crystal form with a melting point of 129-132 °C was reported, later a higher melting modification was found\(^{19,20}\), for the chemical structure see figure 6.

![Figure 6: Chemical structure of benzoine picrate.](image)

An extraordinary fact was that once the stable polymorph was prepared in a laboratory the metastable polymorph could not be isolated anymore. This turned out to be possible again only if the laboratory was cleaned very thoroughly. Another example is xylitol, used as a sweetening agent in tablets, coatings and as substitute for sucrose in foods. Two polymorphs were known, a metastable monoclinic form with a melting point of approximately 61 °C and a stable orthorhombic form with a melting point of 93-94.5 °C\(^{21,22}\), for the chemical structure see figure 7.

![Figure 7: Chemical structure of Xylitol](image)

Once a sample of the orthorhombic form was present in a laboratory in which the monoclinic form was prepared, this form transformed to the stable orthorhombic form on exposure to the air of the laboratory\(^{22}\). It was also not possible anymore to prepare the metastable form in these laboratories. Obviously the seeding effect of nuclei of the stable polymorph plays an important role in the crystallization process.

A more recent example is ritonavir, a medicine which is used to treat AIDS\(^{23}\), for the chemical structure see figure 8.
The new drug application (NDA) for ritonavir was filed in 1995 and the medicine was introduced on the market in 1996. In 1998 some batches failed the dissolution test and a closer investigation revealed that a new and thermodynamically more stable polymorph was formed. In laboratories where samples containing the new polymorph were investigated, it was not possible to prepare the old polymorph anymore. A new formulation of the drug had to be developed. Later it proved to be possible to develop processes to control the polymorph formation. In the light of Ostwald’s step rule it is likely, but not certain, that a newly emerged polymorph is thermodynamically more stable than the previous one. The phenomenon of a seemingly spontaneous replacement of a polymorph for a more stable polymorph is called “disappearing polymorphs”. Despite the fact that a certain polymorph disappeared, it is generally assumed that any polymorph once obtained can be obtained again; it is only a matter of finding the right conditions.

1.4 Form versus habit

In the crystallographic literature two terms are often used to describe crystals; form and habit. Form refers to the internal structure of the crystal, therefore polymorphic forms or simply polymorphs involve different crystal structures of the same chemical entity. Habit refers to the shape of a particular crystal and depends on the growth rates along different directions of the crystal. Unfortunately the use of the terms form and habit in the literature is sometimes ambiguous.

It is important to stress the fact that differences in habit, or morphology, of a crystal do not necessarily imply a change in the crystal structure or polymorphic form.
Morphology is the result of a combination of the way crystals are packed i.e. the polymorphic form, and the conditions during the crystallization process. Morphology plays an important role in the pharmaceutical industry; it can have an effect on the bioavailability and on the processability of the active pharmaceutical ingredient (API).

![Figure 9: Scanning electron microscopy images showing the morphology difference between polymorphs I and II of Venlafaxine hydrochloric acid salt](image)

Bioavailability of an API is among others determined by the dissolution rate. Other examples of processes that are influenced by the morphology of the crystals are filtering, drying and tabletting. In the pharmaceutical industry sphere like crystals are mostly preferred.

During the last century several methods have been developed to predict the crystal morphology. Some well known methods have been developed by Bravais, Friedel, Donnay and Harker (BFDH)\(^{24,25}\), Hartman and Perdok\(^{26, 27, 28}\) and later by Hartman and Bennema\(^{29}\). The availability of fast computers has facilitated the use of numerical modelling techniques to study crystal growth. The program Monty is a development for which Monte Carlo growth simulations can be performed for any crystal structure in any crystallographic orientation as a function of the driving force for crystallization\(^{30}\); for an example see figure 10.

In models for crystal growth the step free energy \(\gamma_{\text{step}}\) plays an important role during birth and spread growth as well as for spiral growth. To combine the importance of steps in crystal growth with morphology modelling, recently an automated procedure, Steplift, was developed to find the molecular configuration that makes up a step\(^{31}\).
1.5 Solubility

Knowledge of the solubility of a compound in different solvents is important. For instance chemical engineers need solubility data to design systems for chemical separation and purification processes. Product and reactant solubilities are usually determined before solvent systems are selected for chemical reactions. Several methods have been developed, in the past decades, to predict solubilities\textsuperscript{32, 33, 34}. Different solvents used for crystallization can lead to different polymorphic forms e.g. in case of 5-fluorouracil water as solvent leads to polymorph I and when nitromethane is used polymorph II is obtained\textsuperscript{35}. The outcome of the crystallization is determined by the initial aggregation induced by the solvent used. Moreover, solvates can be formed depending on the solvent used and the conditions applied. Since solubility and drug dissolution rates of individual polymorphs can be different, resorption and bioavailability in the body can also be different\textsuperscript{36}. For this reason the issue of solubility is of paramount importance for the pharmaceutical industry. Recently the role of solvent in the polymorphic outcome of a crystallization process was described\textsuperscript{1}. One of the conclusions was that it is necessary to determine solubility curves and metastable zone widths to be able to control polymorph crystallization.

Interconversion of polymorphs can be accomplished via solvent or gas phase mediated transformation or directly via a solid-solid phase transformation. Depending on the kinetic barriers, the transformation time can range from instantaneous to infinitely long.

Determination of the precise polymorph transition temperature for an enantiotropically related system is often a laborious task. For such a system the solubility of two polymorphs is, however, equal at the transition point. Therefore solubility data can be used to determine the
transition temperature and the stability region of the polymorphs. Assuming that the solution shows more or less ideal mixing behaviour for the solute and solvent, solubility data will show up as linear curves in a van ‘t Hoff plot. Extrapolation of solubility curves that deviate from the ideal curve can, however, lead to erroneous assignments of the transition temperatures and stability regions of the polymorphs. For an example of solubility curves for a monotropic and an enantiotropic polymorphic system see figure 11.

Figure 11: Solubility curves for a monotropic and an enantiotropic polymorphic system. The solubility is plotted versus the temperature.

1.6 Outline of the thesis

The work presented in this thesis is mainly focussed on the polymorphic behaviour of Active Pharmaceutical Ingredients; Venlafaxine and Ondansetron in particular.

After a general introduction of the phenomenon of polymorphism in chapter 1, a general thermodynamic theory of solubility of molecular crystals is reviewed with the emphasis on solutes showing polymorphism is given in chapter 2. In the third chapter a study of the solubility behaviour and the thermodynamic relations between the different forms of the free base of Venlafaxine is given. For Venlafaxine, which is used as an antidepressant, two polymorphs were known and the thermodynamic relation between the two forms was poorly understood. It was found that an enantiotropic relation exists for all forms. The solubility in heptane, toluene and methanol shows a large deviation from ideal behaviour. It was demonstrated that the deviations are to a large extent determined by the temperature dependence of the difference in fusion enthalpy of the undercooled melt and the solid. In chapter 4 the topotactical solid-solid transition from a racemate to a racemic conglomerate of two forms of the free base of Venlafaxine is described. In chapter 5 the results of a morphology prediction of the three forms of Venlafaxine, using three different methods, is presented. A comparison with the experimentally observed
morphologies is made. The results are semi-quantitative for form I and II. In case of form III the overall shape is reproduced but the experimentally observed indices do not match with the predicted ones. The polymorphism of Ondansetron is studied in chapter 6. Together with the elucidated crystal structure of a vapour grown crystal and the results of molecular modelling, X-Ray Powder Diffraction and solid state Nuclear Magnetic Resonance experiments it was found that the different forms of Ondansetron are in fact locally ordered solid solutions of enantiomers.
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Chapter 2
SOLUBILITY OF MOLECULAR CRYSTALS
- POLYMORPHISM IN THE LIGHT OF SOLUBILITY THEORY -

Abstract

The thermodynamic theory of solubility of molecular crystals in solvents is reviewed with an emphasis on solutes showing polymorphism as in case of many pharmaceuticals. The relation between solubility and binary phase diagrams of the solute solvent system is treated. The astonishing variety of possible solubility curves as a function of temperature is explained using simple models for the solution thermodynamics assuming no mixing between the solvent and solute in the solid phase, though including the case of solvates or pseudo polymorphs. In passing a new method is introduced that allows to estimate the transition temperature of enantiotropically related polymorphs from melting temperatures and enthalpies of the polymorphs.
2.1 Introduction

The first practical understanding of solubility dates back to 1900-1930\textsuperscript{1,2,3}. Since the introduction of the concept of a regular solution by Hildebrand in 1929\textsuperscript{3}, later resulting in the solubility parameter and Scatchard-Hildebrand theory of regular solutions, numerous new models have been proposed in order to describe the non-ideal behavior of solutions more correctly. Predictive methods can be very useful tools to reduce the amount of experiments needed to determine solubilities, and the development of reliable, transferable and quick methods is in continuous progress\textsuperscript{4,5}. Although it might seem as if 1930 is long ago, the theories described before that date still contain the essentials of current solubility models. Modern models have astonishing capabilities, but they are not complete and experimental data are still needed. Especially for multicomponent mixtures, however, experimental methods are not only time-consuming\textsuperscript{4}, but expensive and difficult as well.

Compared to vapor liquid equilibria (VLE), much less attention has been given to SLE (solid liquid equilibria) and solubility, one of the reasons being the much larger importance of rectification as compared to crystallization\textsuperscript{5,6}. Still, SLE and solubility of solids in liquids are of great interest: crystallization processes are used, e.g. for the separation and purification of thermo labile compounds or isomeric compounds with a very similar vapor pressure\textsuperscript{7}. Moreover, the link between solid phase diagrams and solubility is rarely recognized in literature and certainly not generally known. Of course, for solubility behavior showing complete eutectic behavior, it is not so relevant to make this link, but when polymorphism occurs, phase diagrams are becoming useful. Especially for pharmaceutical compounds both fields, solubility of organic compounds in various solvents and polymorphism, have attained an increasing interest in recent years as both are essential, for instance during the development of a new drug\textsuperscript{8}.

It is the aim of this paper to make the connection between (binary) phase diagrams and solubility starting from simple thermodynamics relevant for the solubility behavior of solids in liquids and to apply it to the solubility of polymorphic forms. In that light it is not the aim to describe predictive models for the solubility of molecular crystals but rather to use simple models to describe the thermodynamics behind the astonishing variety of solubility curves found in practical situations. The relation with polymorphism of molecular crystals will be emphasized. Starting from the simplest of all models, the ideal solution model, the complexity of the models will be gradually increased, in all cases limiting the analysis to the essentials. The need for quantitative solubility data, on the other hand, has led to a variety of models for
predicting solubility, each with its specific emphasis; in the following a short overview is given. The first widely applicable predictive model for solubility and solutions is the regular solution theory\textsuperscript{4}. This method makes use of a single solubility parameter, which can describe the real behavior of mixtures of non-polar compounds\textsuperscript{9}. A disadvantage is that the regular solution assumptions cannot describe mixtures of polar molecules, and although some empirical modifications can extend the use of the regular solution model\textsuperscript{10} (a well known example is the Hansen solubility parameter approach\textsuperscript{11}), it is in many cases replaced by more sophisticated methods. Nowadays, the modified UNIFAC(Do) method\textsuperscript{12} is in general the most used method available for predictions. In 1978 already it has been shown that the original UNIFAC is capable of predicting SLE and solubility\textsuperscript{13}, but only for a limited number of compounds. Originally, UNIFAC, being based on a large database of thermodynamic data, was developed for VLE in the temperature range 290-400 K and predictions for SLE or below 290 K can lead to poor results. To increase the temperature range, in modified UNIFAC(Do) the temperature dependence was incorporated and SLE data were used as supporting information, improving the results considerably\textsuperscript{12}. For simple compounds, modified UNIFAC(Do) is a helpful tool for predicting solubility\textsuperscript{14}, but for more complicated molecules, other models have to be used. An example from the pharmaceutical field is the mobile-order-disorder theory, a relatively unknown method that can describe solubility behavior of complex drug based molecules\textsuperscript{8}. Another upcoming approach is predicting real solution behavior with the help of quantum-chemical methods, such as COSMO-RS\textsuperscript{14}. Such models can describe more systems than database-limited methods such as UNIFAC, but they are not yet sophisticated enough for accurate applications\textsuperscript{14,15}.

Despite the advances that have been made, the predictive capability for solubility is still limited. UNIFAC and modified UNIFAC(Do), for example, both make use of equations derived for eutectic systems and although most SLE are indeed eutectic\textsuperscript{16}, systems with for example peritectic behavior cannot be described. The main reason for this limitation is that these models have been developed for temperature ranges relevant for VLE phase diagrams; extension to solid, solid-liquid or solid-solid behavior is difficult. Little attention has been given to SLE or solubility compared to VLE, but still much less attention has been given to the mixing properties in solids.

In the following sections first the link between the thermodynamics of solubility and SLE phase diagrams will be treated. Using the thermodynamic basis underlying these phase diagrams a number of models, which highlight various solubility curves one can encounter, will be analyzed. Finally, the models will be generalized, to include the cases of polymorphism and solvates.
In all cases equilibrium phase diagrams are treated, that is, kinetic effects have been neglected. Especially for crystallization using fast cooling, kinetics cannot be neglected. In recent work, Los et al. have developed methods to determine kinetic phase diagrams. For that the reader is referred to\textsuperscript{17,18,19}.

For solutes that have a chiral center, which is of indisputable relevance for pharmaceuticals, the reader is referred to the book by Jacques et al.\textsuperscript{20}.

### 2.2 The theory of solubility

In this section the thermodynamic background of solubility theory is treated. A rigorous thermodynamic derivation of the solubility of a solid phase in a solvent up to the case of (quasi) regular solutions is given. For the latter a mean field model is used. Special emphasis is put on the relation between solubility curves and phase diagrams.

#### 2.2.1 Solubility and phase diagrams

To discuss the relation between solubility curves and S(olid)-L(iquid) phase equilibria first a typical S-L phase diagram is discussed briefly. For a treatment of the thermodynamics of phase diagrams in general the reader is referred to the excellent book by Stølen and Grande\textsuperscript{21}. The discussion is limited to the case of binary mixtures of compounds A and B, without loss of generality. When these compounds, besides in the liquid phases, also mix in the solid phases, a situation which is in contrast with the case of solubility to be treated in the main body of the present paper, the simultaneous presence of a solid and a liquid phase in thermodynamic equilibrium implies the chemical potentials in these phases to be equal for both components:

\begin{align}
\mu_A^s &= \mu_A^l, \\
\mu_B^s &= \mu_B^l,
\end{align}

where \( \mu \) is the chemical potential and \( l \) and \( s \) label the liquid phase and the solid phase, respectively. In terms of the activity \( a \) of the components A and B these equations become

\begin{align}
\mu_A^s + RT \ln a_A^s &= \mu_A^l + RT \ln a_A^l, \\
\mu_B^s + RT \ln a_B^s &= \mu_B^l + RT \ln a_B^l,
\end{align}
where $\mu^*$ is the chemical potential of the pure compound. In these equations the implicit variables $T$, $P$ and the compositions of the phases are omitted for convenience. Throughout the text it is assumed that the pressure is constant, say $P = P^\circ$, that is, standard pressure and the temperature is considered as a variable.

**S-L ideal phase diagrams**

First the situation is considered for which the mixing is ideal both in the liquid and in the solid phase. This implies that in equations (3) and (4) the activities can be replaced by the mole fractions ($a_i^p = x_i^p$; $i = A, B$; $p = s, l$). Then, both in the liquid and in the solid phase the components A and B can be mixed in any ratio. For the solid phase this leads to a so-called solid solution.

The resulting phase diagram is given in figure 1.

![Figure 1: Typical S-L phase diagram of a binary mixture, ideally mixing in both phases. The hatched area is forbidden because of Gibbs' phase rule; there the lever rule applies.](image)

The temperature $T$ and the composition $x_B = 1 - x_A$ are the variables at the chosen pressure. At high temperatures one finds the well-mixed liquid phase, with no solid phase present. In this region the chemical potential of the solid phase is larger than that of the liquid phase for both compounds ($\mu_i^s > \mu_i^l$; $i = A, B$). The stability domain for only a liquid phase is bounded from below by the liquidus line connecting the fusion temperatures of both (pure) components. Below the liquidus a solid solution starts being formed in combination with the
liquid phase. In equilibrium, the compositions of these two phases follow the liquidus and the solidus (lower limiting line of the hatched area), respectively on the right-hand and left-hand side of the hatched area. This implies that a composition in the hatched area is never realized\(^1\). The well-known lever rule determines the amounts of the liquid and solid phases. The size of the hatched area is determined by the fusion entropies and the difference in fusion temperatures of the two (pure) compounds. Below the solidus only the solid solution is present \((\mu_i^* < \mu_i'; i = A, B)\). The ideal mixing phase diagram of figure 1 is typical for metals or semiconductors, for which the fusion entropies of the pure compounds are usually comparable. For organic crystals, even in case of ideal mixing behavior, the melting entropies can be quite different, leading to a more pronounced phase diagram. Figure 2 shows an example of such a phase diagram, for which the two compounds still mix in all proportions in both phases.

\[ \text{Figure 2: Typical S-L phase diagram of a binary mixture for which the mixing is ideal, but the melting entropies of the pure compounds differ considerably. The hatched area is forbidden because of Gibbs' phase rule; there the lever rule applies.} \]

\textit{S-L eutectic phase diagrams}

Next the case that the compounds A and B are well miscible in the liquid phase but limited miscible in the solid phase, as a result of a relatively large positive enthalpy of mixing in the solid phase, is considered. This leads to solid solutions that are either rich in A, which are

\(^1\)Gibbs' phase rule, \(F = C - P + 2\), for a two component system \((C = 2, \text{ namely } A \text{ and } B)\) and the presence of two phases \((P = 2, \text{ namely } s \text{ and } l)\) implies that for a chosen pressure \(P\) equilibrium is described by a curve in the \(\left(x_B, T\right)\)-diagram. As a consequence, in the hatched area two phases are present.
denoted as α or rich in B, denoted as β. In figure 3 the resulting eutectic phase diagram is drawn. For the eutectic composition, \( x_E \), the liquid phase solidifies at \( T_E \) to form both solid solutions. For temperatures below the eutectic temperature, \( T_E \), only two solid solution phases, α and β, are present each following the solidi. On the right-hand side of the solidus of compound B the system consists of a single solid solution (β) phase. The area on the left-hand side of the solidus of the compound A represents a single α phase. Note, that in the latter two areas, because of the presence of a single phase, Gibbs' phase rule allows both T and \( x_B \) as variables. In the hatched areas, again, the lever rule determines the amounts of the liquid and solid phases with compositions determined by the liquidus and solidi bordering these areas.

![Figure 3: S-L phase diagram of a binary mixture completely miscible in the liquid phase but almost immiscible in the solid phase. The hatched areas are forbidden because of Gibbs' phase rule; there the lever rule applies. Phase α is a solid solution rich in A and β is a solid solution rich in B.](image)

### 2.2.2 Solubility phase diagrams

Now, consider a solvent A in which a solute B is dissolved. A solubility curve is the curve \( x(T) \) that describes the maximal amount of solute B that can be dissolved in the solvent A at a temperature \( T \). In case of a typical solubility problem the fusion temperature of the solvent, \( T_{fus}^A \), is relatively low as compared to that of the solute, that is, the solvent does not solidify within the
temperature range relevant for the solution. Moreover, the solvent, in most cases, does not mix with the solute in its solid phase\textsuperscript{2}. For such systems the phase diagram looks like that in figure 4.

\textbf{Figure 4: Typical S-L phase diagram of a binary mixture well miscible in the liquid phase but immiscible in the solid phase. The hatched area is forbidden because of Gibbs' phase rule; there the lever rule applies. The dashed part of the liquidus of the solute B represents the solubility curve described in the present paper.}

In this figure the solubility curve, which is nothing else than the liquidus line, is drawn as a dashed line. The solidus line of the solute is running along the $x_B = 1$ axis because of the assumption that the solid phase does not include solvent molecules. The solidus of the solvent is not drawn as it is not relevant because it is assumed that the temperature is always far above the fusion temperature of the solvent, $T_{\text{fus}}^A$. As a result, the solid phases are pure phases, either of compound A or B, denoted as $s_A$ and $s_B$, respectively. Note, that the position of the eutectic composition, $x_E$, does not necessarily lie close to the $x_B = 0$ line. In terms of the phase diagram of figure 4, the present paper aims to describe the solubility curve for temperatures well above the eutectic temperature $T_E$, indicated as the dashed liquidus in the figure. Thus, the solubility curve is nothing else than the line that in a $T(x)$ phase diagram divides the phase region consisting of a liquid mixture of A and B, for which no solid phase is present, and the region where a liquid mixture of A and B is present together with a pure solid phase of the solute B. In other words, thermodynamic equilibrium for a saturated solution is achieved when

\textsuperscript{2} Solvates are an exception to that; in the case of solvates the composition of the solid phase is fixed.
the solid phase B is in contact with a saturated solution at a given temperature $T$ and pressure $P$. Equilibrium along the solubility curve implies that the chemical potential of the solute in the solid phase is equal to that of the solute in the liquid phase. In other words, thermodynamic equilibrium as described by equations (3) and (4), for the case of a solubility curve is determined by

$$\mu^*_{B} = \mu^*_{B} + RT \ln a^*_B, \quad (5)$$

as the presence of a solid phase of the solvent A is not considered and the pure solid phase implies $a^*_B = 1$. In terms of the activity coefficient $\gamma^*_B$ and the mole fraction $x^*_B$ of the solute in the solution equation (5) becomes

$$\mu^*_{B} = \mu^*_{B} + RT \ln \gamma^*_B x^*_B = \mu^*_{B} + RT \ln x^*_B + RT \ln \gamma^*_B, \quad (6)$$

where the label $l$ in the right-hand terms is omitted for convenience. This expression can be rearranged to find the solubility of the solute in terms of its mole fraction $x^*_B$:

$$\ln x^*_B = \frac{\mu^*_{B} - \mu^*_{B}}{RT} - \ln \gamma^*_B \quad (7)$$

The second term on the right-hand side of eq. (7) involves the generally very complex chemistry of the interactions between the solute and the solvent molecules. The first term on the right-hand side involves only properties of the pure solute, for which the difference in chemical potential can be described in terms of the Gibbs free energy difference (per mol) $g^*_{B} = \frac{G^*_B}{n}$, where $n$ is the total number of moles in the system, according to

$$\frac{\mu^*_{B} - \mu^*_{B}}{RT} = \frac{g^*_{B} - g^*_{B}}{RT} = \frac{\Delta_{x\to\gamma} g^*_B(T)}{RT} \quad (8)$$

The Gibbs free energy $\Delta_{x\to\gamma} g^*_B(T)$ at the system temperature is not easily determined experimentally. Usually a thermodynamic cycle that is depicted in figure 5 is used for that\textsuperscript{22,23,24}. 

- 27 -
Figure 5: Alternative thermodynamic cycle for the transition from the solid state of the solute to its undercooled liquid state at temperature $T$.

In this figure, as an alternative for the direct path $1 \rightarrow 4$ which involves $\Delta_{s \rightarrow l} g_B^*(T)$, the solute is heated from the system temperature to its fusion temperature $(1 \rightarrow 2)$, transformed to the liquid phase $(2 \rightarrow 3)$ and subsequently cooled down in the liquid state back to the system temperature $(3 \rightarrow 4)$, resulting in the supercooled liquid. Conservation of free energy implies

$$
\Delta_{s \rightarrow l} g_B^*(T) = \Delta_{l \rightarrow 4} g_B^*(T) = \Delta_{1 \rightarrow 2} g_B^* + \Delta_{2 \rightarrow 3} g_B^* + \Delta_{3 \rightarrow 4} g_B^* \tag{9}
$$

As at the melting temperature $T_{\text{fus}}$ the liquid and solid phase are in thermodynamic equilibrium it follows that

$$
\Delta_{2 \rightarrow 3} g_B^* = \Delta_{\text{fus}} g_B^*(T_{\text{fus}}) = 0 \tag{10}
$$

Combining equations (8) to (10) results in

$$
\frac{\mu_B^* - \mu_B^*}{RT} = -\int_{T_{\text{fus}}}^{T} d\left(\frac{g_B^*}{RT}\right) - \int_{T_{\text{fus}}}^{T} d\left(\frac{g_B^*}{RT}\right) - \frac{1}{R} \int_{T_{\text{fus}}}^{T} d\left(\frac{\Delta_{s \rightarrow l} g_B^*}{T}\right) \tag{11}
$$

\[^3\] Prausnitz consequently uses the triple point temperature $T_{\text{triple}}$ instead of the fusion temperature because of the possible presence of a gas phase, implying a system pressure equal to the triple point pressure.

\[^4\] If a solid state phase transition occurs before the melting point the Gibbs free energy change of that phase transition should be added in the cycle of figure 5; this is the case for a solute showing polymorphism which is treated in section 3. In addition, if the solid sublimes before melting the sublimation transition should be used instead of the fusion transition.
In this equation $T'$ is used as a dummy variable for the integration to avoid confusion with the system temperature $T$. Because the system is assumed to be at constant pressure $P$ one can, subsequently, use the Gibbs-Helmholtz equation to rewrite expression (11) to

$$
\frac{\mu_L^* - \mu_B^*}{RT} = -\frac{1}{R} \int_{T}^{T_fus} \left( \frac{\Delta_{s-sl} h_B^*}{(T')^2} \right) dT'.
$$

To obtain experimental values for this difference in chemical potential of the pure solute the transition enthalpy $\Delta_{s-sl} h_B^*$ has to be measured between the system temperature $T$ and the melting temperature $T_{fus}$, which is still difficult but, in principle, more accessible than measuring the transition Gibbs free energies from equation (8) as $\Delta_{s-sl} h_B^*$ can be determined by measuring the heat of solidification of supercooled melts at various temperatures. If equation (13) is combined with equation (7) one finds a general expression for the solubility of a solid phase $B$ in a solvent $A$ at temperature $T$ and constant pressure $P$, under the assumption that the amount of $A$ dissolved in the solid phase $B$ is negligible, reading

$$
\ln x_B = \frac{1}{R} \int_{T}^{T_fus} \left( \frac{\Delta_{s-sl} h_B^* (T')}{(T')^2} \right) dT' - \ln \gamma_B ,
$$

where $\Delta_{s-sl} h_B^* (T') = h_B^* (T') - h_B^* (T_fus)$.

In appendix A some approximations for the first term on the right-hand side of equation (14) are discussed. The second right-hand term in this expression, $\ln \gamma_B$, which describes the thermodynamics of the solute dissolved in the solvent as a deviation from ideal mixing, is generally rather involved. To include the effect of mixing the undercooled liquid phase of the

---

5 The Gibbs-Helmholtz equation, strictly spoken, only holds for equilibrium situations, that is, at $T_{fus}$; here, it is assumed that the equation also holds for $T < T' < T_{fus}$.
solute B with the solvent A in the scheme of figure 5 the path has to be extended. The additional path $4 \to 5$ is drawn in figure 6.

\[ \Delta_{1 \to 4} \mu_B + \Delta_{4 \to 5} \mu_B = (\mu_B^* - \mu_B^*) + \Delta_{\text{mix}} \mu_B = 0 \]  

(15)

Describing the equilibrium between the solid solute and the dissolved solute. Comparison with equations (5) and (13) leads to

\[ RT \ln \alpha_B = RT \ln x_B + RT \ln \gamma_B = \Delta_{\text{mix}} \mu_B = -T \int_T^{T_{\infty}} \left( \frac{\Delta_{\text{mix}} H_B^*}{T} \right) dT. \]  

(16)

From a thermodynamic point of view there is an essential difference between mixtures of liquid phases or solid phases in equilibrium with their vapor mixtures and the solubility of a solid compound in a solvent. This becomes clear when one compares the mixing thermodynamics of an undercooled liquid phase of a solute in a solvent, which will be treated further on, with the mixture of two liquids. In the latter case one can establish thermodynamic equilibrium by mixing the two (or more) liquids by equating the chemical potentials of all components with their chemical potentials in the gas phase as is usually done for (L)iquid-(V)apor phase diagrams. For determining the solubility of a solid phase in a solvent, however, the mixing term depends, in general, not on the gas phase chemical potential (see footnote I). In figure 6, one can not speak of an equilibrium between the undercooled solute and the solvent; only the final solution and the solid phase can be in thermodynamic equilibrium. This is expressed by equation (5) for the
solute B. In that light the present models are a limited subset of the situations modeled by Pelton and Thompson. In the latter paper the phase diagram of a binary system is studied in dependence of the mixing parameters both of the liquid and of the solid (solution) phase; in other words of solid-liquid equilibria.

In the following sections three models for the mixing term will be considered in more detail, namely ideal mixing, a regular solution model and a quasi regular solution model. An important reference model is that of the ideal solution for which there is no enthalpy of mixing ($\Delta_{mix}^H = 0$) and a mere mixing entropy contribution $\Delta_{mix}^S$ representing the ideal configurational entropy of mixing. All deviations of this ideal solution model are covered by so-called excess contributions, defined as

$$
\Delta_{mix}^E G = \Delta_{mix}^E H - T \Delta_{mix}^E S \equiv \Delta_{mix} G - \Delta_{mix}^H \Delta_{mix}^S, \text{ or }
$$

$$
\Delta_{mix}^E H \equiv \Delta_{mix}^H \text{ and } \Delta_{mix}^E S \equiv \Delta_{mix} S - \Delta_{mix}^H \Delta_{mix}^S.
$$

In table 1 an overview of the models is given.

**Table 1:** The various approximations for the mixing enthalpy and entropy considered in the present paper; the athermal and general situation will not be discussed in detail.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta_{mix}^H$</th>
<th>$\Delta_{mix}^S$</th>
<th>$\Delta_{mix}^E H$</th>
<th>$\Delta_{mix}^E S$</th>
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<td>regular</td>
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<td>$\Delta_{reg}^H$</td>
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<td>$\Delta_{qu-reg}^S$</td>
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<tr>
<td>quasi regular</td>
<td>$\Delta_{reg}^H$</td>
<td>$\Delta_{ideal}^S + \Delta_{qu-reg}^S$</td>
<td>$\Delta_{reg}^H$</td>
<td>$\Delta_{qu-reg}^S$</td>
</tr>
<tr>
<td>general</td>
<td>$\Delta_{mix}^E H$</td>
<td>$\Delta_{ideal}^S + \Delta_{mix}^E S$</td>
<td>$\Delta_{mix}^E H$</td>
<td>$\Delta_{mix}^E S$</td>
</tr>
</tbody>
</table>

### 2.2.3 Ideal solutions

Ideal solutions already offer a lot of insight in the problem of solubility. For an ideal solution, the solubility curve is the liquidus of the solute B of a phase diagram of two compounds A and B that are immiscible in the solid phase and perfectly miscible in the liquid phase. On a
molecular level this implies that the reaction energy for the mixing reaction \( \phi_{AA} + \phi_{BB} \rightarrow 2\phi_{AB} \) between the solute and solvent molecules is zero

\[
\phi_{AB} - \frac{1}{2}(\phi_{AA} + \phi_{BB}) = 0
\] (19)

where \( \phi_{AA} \) is the interaction energy between the solvent molecules, \( \phi_{BB} \) between the solute molecules and \( \phi_{AB} \) represents the heterogeneous interaction energy. In other words, \( \Delta_{\text{mix}}^\text{ideal} H = 0 \).

Then, the only contribution of the mixing to equation (16) is an ideal configurational entropic term, \( \Delta_{\text{mix}}^\text{ideal} S \), as a result of the mixture of \( n_A \) mol of solvent and \( n_B \) mol of the (supercooled) liquid solute. The total Gibbs free energy of mixing is\(^{26}\)

\[
\Delta_{\text{mix}}^\text{ideal} G = -T \Delta_{\text{mix}}^\text{ideal} S = nRT \left( x_A \ln x_A + x_B \ln x_B \right),
\] (20)

where \( n = n_A + n_B \). The ideal mixing contribution to the chemical potential change of the solute can be found by differentiating equation (20) with respect to \( n_B \) resulting in

\[
\Delta_{\text{mix}}^\text{ideal} \mu_B = \left( \frac{\partial \Delta_{\text{mix}}^\text{ideal} G}{\partial n_B} \right)_{T,P,n_A} = RT \ln x_B
\] (21)

Substituting this result in equation (16) one obtains for the activity coefficient \( \gamma_B = 1 \). Using equation (15) this leads to the ideal solubility equation

\[
\ln x_B^\text{ideal} = \frac{\mu_B^* - \mu_{B}^{*\text{eq}}}{RT} = -\frac{1}{R} \int_{T}^{T^*} \Delta_{x=x_0} h_B^* \left( T' \right) \left( T' \right)^2 dT'.
\] (22)

Note, that this solubility equation for an ideal solution only depends on pure solute parameters.

**Interpretation of the ideal solubility curve**

As long as it is assumed that the solution is ideal the solubility curve can be described by equation (22) for temperatures above the fusion temperature of the solvent and compositions
with $x_B > x_E$. In most cases, for which $T_{\text{fus}}^{\text{solv}} << T_{\text{fus}}^{(B)}$, $x_E$ is very small. As mentioned before, therefore, the solubility curve is considered to be represented by the liquidus of the solute for compositions in the range $0 \leq x_B < 1$.

Assuming that the fusion enthalpy of the pure solute is independent of the temperature for all values of interest for $x_B$, equation (22) reduces to

$$\ln x_{\text{ideal}} = - \frac{\Delta_{\text{fus}} h_B^*}{R} \left[ \frac{1}{T} - \frac{1}{T_{\text{fus}}} \right],$$

(23)

Equation (23), when plotted as $\ln x$ versus $1/T$, a representation of solubility curves often referred to as van ’t Hoff curves, leads to a linear solubility curve which will be the reference curve in section 2.4 dealing with regular solutions.

In the limit of very high concentrations, $x_B \approx 1$, the solubility curve can be interpreted as a result of freezing point depression, the solvent acting as an impurity. Note that this approach is an alternative treatment of freezing point depression as it usually is considered for the case of a liquid with a dissolved solid or liquid acting as the impurity. For $x_B \approx 1$ and using $\ln x_B = \ln (1-x_A) = x_A$, equation (23) becomes

$$T_{\text{fus}} - T = \frac{RT_{\text{fus}}^2}{\Delta_{\text{fus}} h_B^*} x_A,$$

(24)

which is the standard equation for the linear freezing point depression, with the solvent acting as the impurity. As the solid is usually dissolved at temperatures $T$ far below the fusion temperature, the approximation used to arrive at equation (24) can usually not be made. In practice, $\Delta_{\text{fus}} h_B^*$ will depend on temperature, as a result of which even for ideal solutions a classical solubility curve of $\ln x_B$ versus $1/T$ does not result in a linear curve. In practice, however, the temperature range studied is rather limited, such that this curve is usually close to linear. In that case the slope of this curve should not be interpreted as $\Delta_{\text{fus}} h_B^*/R$ but rather as $\Delta_{x-B} h_B^* (T_{\text{exp}})/R$, sometimes denoted as $\Delta_{\text{diss}} h_B^*/R$, where $T_{\text{exp}}$ represents the average experimental temperature.
To get an impression of the more general case of an ideal solubility curve the temperature
dependence of $\Delta_{\text{sol}} h^*_B(T)$ in equation (22) is considered in appendix A in terms of a linear
temperature dependence of the molar heat capacity according to

$$\Delta_{\text{sol}} c_p(T) = c_p^f(T) - c_p^s(T) = \Delta c_p^0 + \Delta c_p^1(T - T_{\text{fus}}).$$  \hspace{1cm} (25)$$

Here the case for which $\Delta c_p^1 = 0$ will be considered. Then equation (51) becomes

$$\ln x_B = \frac{\Delta_{\text{fus}} h^*_B}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] + \frac{\Delta c_p^0}{R} \left[ \frac{T_{\text{fus}}}{T} - \ln \frac{T_{\text{fus}}}{T} - 1 \right].$$  \hspace{1cm} (26)$$

This equation is plotted for a few values for $\Delta c_p^0$ in figure 7. The common point of the
solubility curves in this figure is the fusion temperature of the pure solute, marked by the dashed
black lines. For the limiting case that $T = T_{\text{fus}}$, equation (26) reduces to $\ln x_B = 0$, that is $x_B = 1$,
corresponding to the pure liquid solute. The straight black lines in the van ‘t Hoff plots (figures
7c and d) represent the ideal solubility situation for $\Delta c_p^0 = 0$. The red curves in figure 7 represent
the situation for which $\Delta c_p^0 > 0$. In that case the solubility is larger than the reference straight line,
showing a minimum solubility for a temperature given by

$$T_{\text{min}} = T_{\text{fus}} - \frac{\Delta_{\text{fus}} h^*_B}{\Delta c_p^0} \left( \Delta c_p^0 > 0 \right)$$  \hspace{1cm} (27)$$

The blue curves show the situation for $\Delta c_p^0 < 0$, resulting in a lower solubility and a non-linear
van ‘t Hoff curve as a result of the increasing contribution of the $\Delta c_p^0$ term in equation (26)
with decreasing temperature. A negative value for $\Delta c_p^0$ is quite rare; an exception might have
been found for pyrene$^{28}$. Usually, $c_p^f(T) > c_p^s(T)$ for temperatures below the fusion temperature.
Figure 7: The solubility curves according to equation (26) for a) $\Delta_{\text{fus}} h^*_B = 20 \text{ kJ/mol}$ and $T_{\text{fus}} = 370 \text{ K}$ and b) $\Delta_{\text{fus}} h^*_B = 100 \text{ kJ/mol}$ and $T_{\text{fus}} = 570 \text{ K}$. The dashed black lines indicate $T_{\text{fus}}$. The heavy black lines represent the ideal solubility case for which $\Delta c^0_p = 0$. The red lines show the values $\Delta c^0_p = 100$, 200 and 500 J/mol K and the blue lines $\Delta c^0_p = -100$ and -200 J/mol K, respectively. The figures (c) and (d) show the corresponding van't Hoff curves of (a) and (b) respectively.

Although a negative value for $\Delta c^0_p$ is rare, a positive value for $\Delta c^0_p$ large enough to lead to a minimum in the van’t Hoff curve at an accessible temperature can not be ruled out, a priori. For temperatures $T < T_{\text{min}}$, the solubility increases with decreasing temperature. This phenomenon is known as retrograde solubility. The limiting case for which $x_B = 1$ below the fusion temperature, obviously, is non-physical. To get an idea of the quantitative effect of
\[ \Delta_{s-m}c_p(T) \] on the solubility curve the case of a pharmaceutical compound, venlafaxine, is considered in detail in appendix B.

In conclusion, in almost all cases \( \Delta_{s-m}c_p(T) > 0 \), leading to an increase in solubility and a deviation from the linear van’t Hoff curve. For large positive values a minimum in the solubility can occur. Equation (27) can be used as an indication for such behavior. There are, however, other causes of retrograde solubility. In section 2.6 the issue of retrograde solubility will be touched upon in more detail.

### 2.2.4 Regular solutions

As mentioned before, approximations for the solubility, or in general mixing of phases, beyond the ideal case are expressed in terms of excess contributions, like \( \Delta_{mix}^E G, \Delta_{mix}^E H \) and \( \Delta_{mix}^E S \), as in equations (17) and (18). Accordingly, the excess mixing chemical potential difference is given by

\[
\Delta_{mix}^E \mu_B = \left( \frac{\partial \Delta_{mix}^E G}{\partial n_B} \right)_{T,P,n,A} = \Delta_{mix} \mu_B - \Delta_{mix}^{ideal} \mu_B = RT \ln \gamma_B. \tag{28}
\]

Regular solutions\(^6\) are a special case defined by the restriction that \( \Delta_{mix}^E H \neq 0 \) and \( \Delta_{mix}^E S = 0 \). On a molecular level this restriction implies that for the mixing reaction \( \phi_{AA} + \phi_{BB} \rightarrow 2\phi_{AB} \), it holds that \( \phi_{AB} \neq \frac{1}{2}(\phi_{AA} + \phi_{BB}) \). For the limitations of regular solution models the reader is referred to Prausnitz et al.\(^9\).

The regular solution model treated here is based on the mean field model. In the mean field model it is assumed that the occurrence of the various interactions in the solution, \( \phi_{AB}, \phi_{AA} \) and \( \phi_{BB} \) is randomly distributed, independent of the values of the interaction energies\(^7\). To simplify the statistics of the neighboring interactions further, the solvent is divided in cells which are either filled with a solute molecule B or a solvent molecule A. Each cell has \( Z \) interactions, with neighboring cells. Depending on the concentration of solute \( x_B \) the number of AB

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\(^6\) The term regular solutions was first introduced by Hildebrand\(^34\) and further restricted by Prausnitz et al.\(^9\).

\(^7\) This assumption will obviously be too crude for situations where \( \phi_{AB}, \phi_{AA} \) and \( \phi_{BB} \) differ too much.
interactions, each having an energy \( \phi_{AB} \), between the cells in the model equals 
\[ Zx_Bx_A = Zx_B(1-x_B). \]
Therefore the total excess enthalpy now becomes

\[
\Delta_{\text{mix}}^E H = \Delta_{\text{mix}}^E H = Znx_B(1-x_B)\left[ \phi_{AB} - \frac{1}{2}(\phi_{AA} + \phi_{BB}) \right],
\]

(29)

where the superscript reg refers to the present mean field model for a regular solution and the molecular interaction energies are chosen to be negative. Defining the excess interaction energy by

\[ \phi^E = \phi_{AB} - \frac{1}{2}(\phi_{AA} + \phi_{BB}) \]

and combining equations (20) and (29) one obtains

\[
\Delta_{\text{mix}}^E G = nx_B(1-x_B)\Delta_{\text{mix}}^E h + nRT(x_A \ln x_A + x_B \ln x_B).
\]

(31)

Differentiating this expression with respect to \( n_B \) one obtains for the chemical potential change of the solute

\[
\Delta_{\text{mix}}^E \mu_B = \left( \frac{\partial \Delta_{\text{mix}}^E G}{\partial n_B} \right)_{T,P,n_A} = \Delta_{\text{mix}}^E h(1-x_B)^2 + RT \ln x_B.
\]

(32)

Substituting this result in equation (16) one obtains for the solubility equation of the regular mean field solution

\[
\ln x_B^{\text{reg}} = -\frac{1}{R} \int T \left( \Delta_x^E h_B^*(T) \right) dT - \frac{\Delta_{\text{mix}}^E h}{RT}(1-x_B^{\text{reg}})^2.
\]

(33)

The activity coefficient in equation (16) is, thus, determined through

\[
\ln \gamma_B^{\text{reg}} = \frac{\Delta_{\text{mix}}^E h}{RT}(1-x_B^{\text{reg}})^2.
\]

(34)
To show the effect of $\Delta_{mix}^{reg}h$ on the solubility it will be assumed that $\Delta_{x\to h}^{\text{reg}}h_B^*$ is independent of temperature, implying

$$\ln x_B^{\text{reg}} = \frac{\Delta_{fus}^{\text{reg}}h_B^*}{R} \left[ \frac{1}{T_{fus}} - \frac{1}{T} \right] - \frac{\Delta_{mix}^{\text{reg}}h}{R} \left[ \frac{(1-x_B^{\text{reg}})^2}{T} \right].$$ (35)

This equation is plotted for a few values for $\Delta_{mix}^{\text{reg}}h$ in figure 8.

Again, the straight black lines in the van ‘t Hoff plots (figures 8c,d) represent the ideal solubility situation described by equation (23). The blue curves show the situation for $\Delta_{mix}^{\text{reg}}h_B > 0$ which can, according to equation (29), be interpreted as $\phi_{AB} > \frac{1}{2}(\phi_{AA} + \phi_{BB})$, a situation which is sometimes referred to as less than equivalent wetting, equivalent wetting referring to the ideal case. The red curves represent the situation for which $\phi_{AB} < \frac{1}{2}(\phi_{AA} + \phi_{BB})$, or more than equivalent wetting. The reduced affinity between the molecules A and B for the blue curves leads to a lower solubility at a given temperature as compared to the ideal solubility curve. The reverse holds for the red curves. In the limit of low concentrations of the solute, that is $x_B \to 0$, equation (35) becomes

$$\ln x_B^{\text{reg}} = \frac{\Delta_{fus}^{\text{reg}}h_B^*}{R} \left[ \frac{1}{T_{fus}} - \frac{1}{T} \right] - \frac{\Delta_{mix}^{\text{reg}}h_B^*}{RT}.$$ (36)

which in the van ‘t Hoff plot leads to straight lines with a slope $(\Delta_{fus}^{\text{reg}}h_B^* + \Delta_{mix}^{\text{reg}}h_B^*)/R$ instead of $\Delta_{fus}^{\text{reg}}h_B^*/R$ for the ideal solubility curve, as can be seen in figure 8c.

According to equation (35) the freezing point depression for small $x_A$ becomes

$$T_{fus} - T = \frac{RT_{fus}^2}{\Delta_{fus}^{\text{reg}}h_B^* x_A} - \frac{\Delta_{mix}^{\text{reg}}h_B T_{fus}}{\Delta_{fus}^{\text{reg}}h_B^*} x_A^2,$$ (37)

showing an extra term as compared to equation (24).
Figure 8: The solubility curves according to equation (35) for a) $\Delta_{\text{mix}}^{\text{reg}} h_B^* = 20$ kJ/mol and $T_{\text{fus}} = 370$ K and b) $\Delta_{\text{mix}}^{\text{reg}} h_B^* = 100$ kJ/mol and $T_{\text{fus}} = 570$ K. The dashed black lines indicate $T_{\text{fus}}$. The heavy black lines represent the ideal solubility case for which $\Delta_{\text{mix}}^{\text{reg}} h_B = 0$. The blue lines show the values $\Delta_{\text{mix}}^{\text{reg}} h_B = 1,10$ and 20 kJ/mol and the red lines $\Delta_{\text{mix}}^{\text{reg}} h_B = -1$ and -5 kJ/mol, respectively. The figures c) and d) show the corresponding van 't Hoff curves of a) and b), respectively.

Another important feature in figure 8 is that for large enough positive values of $\Delta_{\text{mix}}^{\text{reg}} h_B$, the solubility curves show a horizontal plateau in the $T$ versus $x$ curves (figures 8 a,b). For these values of $\Delta_{\text{mix}}^{\text{reg}} h_B$ a particular situation occurs. The solubility equation described by equation (23) would show a maximum and a minimum in the $x_B$ interval of the plateau. In between this maximum and minimum, however, the system is not stable due to liquid-liquid, (L-L), separation, for which a diluted solution and a concentrated solution are in equilibrium. As a result, the solubility curve has to be replaced by the horizontal lines in an appropriate interval for
the mole fraction. According to Gibbs' phase rule for a two component system, considered here, three phases can only coexist for a single point in the \((T,x)\) phase diagram. The end points of the horizontal lines correspond to two solutions in equilibrium with the solid phase of the solute. In the van 't Hoff plots the L-L separation temperature corresponds to the vertical part in the blue lines. Often the term oiling out is used for L-L separation\(^{29}\). The occurrence of L-L separation has not been reported often for molecular compounds. For pharmaceuticals this might be explained by the, often, classified information of the data, see e.g. Deneau and Steele\(^{30}\). Papers with full details on this topic were published by Laferrière et al.\(^{31,32}\).

In case of protein crystallization, although strictly spoken not a binary system as a result of the presence of additional salts, L-L separation is a common feature often a result of kinetic effects\(^{33}\). In appendix C the thermodynamics behind the cause of L-L separation are explained; there, also the conditions for L-L separation for the present regular solution model will be given.

For negative values of \(\Delta_{\text{mix}}^{\text{reg}} h_B\) no extrema are found in the solubility curve within the present model.

**2.2.5 Beyond regular solutions**

In the strict definition of a regular solutions as introduced by Hildebrand\(^{34}\) the excess entropy of mixing, \(\Delta_{\text{mix}}^{E} S\), is zero (cf. Table 1). In this section a non-zero term for \(\Delta_{\text{mix}}^{E} S\) will be added to the regular solution model. For that it will be assumed that the excess entropy, like in the mean field model for the regular solution, will depend on the number of heterogeneous interactions between the solvent molecules \(A\) and the solute molecules \(B\), leading to a Gibbs free energy of mixing equal to

\[
\Delta_{\text{mix}}^{\text{qu-reg}} G = nRT \left( x_A \ln x_A + x_B \ln x_B \right) + n x_B \left( 1 - x_B \right) \left[ \Delta_{\text{mix}}^{\text{reg}} h - T \Delta_{\text{mix}}^{\text{qu-reg}} s \right]. \tag{38}
\]

This excess entropy term should therefore be interpreted as being mainly due to a change in the vibrational and rotational degrees of freedom of the molecules, but also containing extra configurational contributions. This model is sometimes referred to as the quasi regular solution model, explaining the superscript in the equation\(^{31}\). Differentiating equation (38) with respect to \(n_B\) one finds analogously to the case of regular solutions, neglecting the temperature dependence of \(\Delta_{x\to \infty} h_B^*(T)\), for the solubility equation
\[
\ln x_B^{\text{qu}-\text{reg}} = \frac{\Delta_{\text{fus}} h_B^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] - \left( \frac{\Delta_{\text{mix}}^{\text{reg}} h_B - T \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S}{R} \right) \left( 1 - x_B^{\text{qu}-\text{reg}} \right)^2. \tag{39}
\]

The activity coefficient in equation (16), for the quasi regular solution model, thus becomes \( \ln \gamma_B = \left( \frac{\Delta_{\text{mix}}^{\text{reg}} h}{RT} - \frac{\Delta_{\text{mix}}^{\text{qu}-\text{reg}} S}{RT} \right) \left( 1 - x_B^{\text{qu}-\text{reg}} \right)^2 \). The resulting solubility curves are plotted in figure 9 for several of the parameters of figure 8b and a realistic value of \( \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S = 10 \text{ J/mol K} \).

In the van 't Hoff plots the slopes for the lower limit of \( x_B \) are still given by \( \left( \frac{\Delta_{\text{fus}} h^* + \Delta_{\text{mix}}^{\text{reg}} h_B}{RT} \right) \) as in the regular solution case. Comparing figures 8b,d and figure 9 shows that positive values of the excess entropy suppress the deviation from ideality of the excess enthalpy, while negative values amplify the deviation.

![Figure 9: The solubility curves according to equation (39) for \( \Delta_{\text{fus}} h^*_B = 100 \text{ kJ/mol} \) and \( T_{\text{fus}} = 570 \text{ K} \). The dashed black lines indicate \( T_{\text{fus}} \). The heavy black lines represent the solubility case for which \( \Delta_{\text{mix}}^{\text{reg}} h_B = \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S = 0 \). The blue lines show the values \( \Delta_{\text{mix}}^{\text{reg}} h_B = 1, 10 \text{ kJ/mol} \) and \( \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S = 0 \). The green curves show the corresponding situations for \( \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S = 10 \text{ J/mol K} \); the red curves for \( \Delta_{\text{mix}}^{\text{qu}-\text{reg}} S = -10 \text{ J/mol K} \). a) the \((x, T)\)-diagrams and b) the corresponding van 't Hoff curves.](image)

A positive and negative value for the excess entropy can be interpreted as an increase, respectively decrease, in vibrational and rotational entropy in the solution as a result of the difference between the A-B interactions as compared to the A-A and B-B interactions. In other words, any excess enthalpy, either positive or negative, will imply a change in excess entropy. In
many cases $\Delta_{\text{mix}}^{\text{reg}} h_B$ and $\Delta_{\text{mix}}^{\text{dimer}} s$ have the same sign$^{35}$ resulting in a mutually compensating effect in equation (39), which in turn leads to a smaller deviation from the ideal solubility curve.

### 2.2.6 Deviations from mean field models

In the previous two sections the solubility of solutes has been discussed on the basis of a mean field model assuming a random distribution of the solute and solvent molecules, with isotropic interaction between them. Relatively strong homogeneous interactions lead to clustering of A or B molecules resulting in deviations from the mean field approximation. The case of hydrogen bonds in solutions and complexes formed in electrolytic solutions are notorious for that. Both situations are not well described by the simple models presented here. More sophisticated models as referred to in the introduction deal with clustering effects, but are beyond the scope of this paper as they depend too much on the specific compound and solvent used. However, the special case of retrograde solubility will be treated briefly in the following section.

### 2.2.7 Retrograde solubility

As was pointed out in section 2.3 retrograde solubility, i.e. decreasing solubility with increasing temperature within a certain temperature interval, can in exceptional cases be explained by a temperature dependence of the difference in heat capacity of the undercooled molten solute and the solid solute. Also the (quasi) regular solution models cannot describe this phenomenon without taking such a difference in heat capacity into account. In general two causes can be distinguished for retrograde solubility.

A difference in heat capacity, $\Delta_{s-s} c_p (T)$, between the undercooled liquid phase and the solid phase of the solute as described in section 2.3 might lead to a retrograde solubility. Although in appendix B it is shown that this effect is not large enough for the case of the pharmaceutical compound venlafaxine, there might exist examples of more complex organic molecules for which $\Delta_{s-s} c_p (T)$ is large enough. Equation (27) shows that a retrograde solubility can be expected in case of a solute that has a relatively small heat of fusion $\Delta_{\text{fus}} h^*$ combined with a large difference in heat capacities $\Delta_{s-s} c_p (T)$. Calculations similar to the ones performed in appendix B, for fat crystals (SSS), e.g., showed a fully negligible effect of the relatively large value of $\Delta_{s-s} c_p (T)$, ranging from 400 J/molK at $T=300$ K to 100 J/molK at $T=T_{\text{fus}}$, caused by the large enthalpy of fusion, $\Delta_{\text{fus}} h^* = 196$ kJ/mol$^{36}$. In general, a strong effect is to be expected for
compounds that have a large number of degrees of freedom in the (undercooled) liquid phase as compared to the solid phase.

A decrease in entropy in the solution as a result of strong solvation interactions can also lead to retrograde solubility. Such an effect should be included in $\Delta_{\text{mix}}^E S$ with an appropriate temperature dependence. Examples are found for aqueous solutions of salts. Small cations like $\text{Li}^+$ and $\text{Ca}^{2+}$ result in large ordered hydration shells leading to strong heterogeneous interactions between the solute and solvent. The same holds for some cases of transition metal ions forming complexes like $\text{Co}^{4+}$ in the salt $[\text{N(CH}_3\text{)}_2\text{CoCl}_4]$. No retrograde solubilities were found to be reported for salts of pharmaceuticals, possibly because in most cases the counter ion is an anion for these salts.

Hexamethylenetetramine (HMT), used in explosives and plastics industries, is an example of a molecular crystal that shows retrograde solubility\(^{37,38}\). The hydrate of the compound ($\text{C}_6\text{H}_{12}\text{N}_4.6\text{H}_2\text{O}$) shows a eutectic behavior in the aqueous phase diagram, which dissolves incongruently at 286 K to form the anhydrate ($\text{C}_6\text{H}_{12}\text{N}_4$). The solubility curve of the anhydrate, although nearly temperature independent, is a retrograde\(^{39}\). As a result of the presence of a hexahydrate, it is tempting to attribute the retrograde behavior to the formation of complexes with the solvent\(^{37}\); lack of thermodynamic data, makes it difficult to determine the effect of the heat capacity difference $\Delta \mu_{\text{sol}} c_p(T)$.

There is another case of retrograde solubility, beyond the scope of this paper. In case of solid solutions the mixing thermodynamics of the solvent in the solute has to be included and one has to consider the equations (3) and (4) instead of equation (5). Even for a regular solution model this leads to an additional term $\Delta_{\text{mix}}^V H^s$, where the superscript $s$ refers to the solid solution phase. This additional parameter is enough to lead to retrograde solubility as was shown for solid solutions of $\text{Sb}$ and $\text{Cu}$ in $\text{Si}$ and $\text{Ge}^{40}$. Such a retrograde solubility curve is visible in figure 3 for the solidus of the solid solution phase $\beta$.

Solid solutions are quite common in alloys and mixtures of similar organic molecules like alkanes or fats\(^{35}\), but rarely observed for molecular crystals like pharmaceuticals dissolved in common solvents. If solid solutions play a role, retrograde solubilities can occur. Solid solutions should not be confused with solvates for which the solvent and solute are built-in in a stoichiometrical relation in the solid phase Solvates, therefore lead to a very different phase diagram as will be discussed in section 3.1.
2.3 Solubility and polymorphism

Polymorphism is the phenomenon that a compound, the solute $B$, can crystallize in more than one crystal structure under seemingly identical conditions. Although there is only one thermodynamically stable phase for a given temperature and pressure, often metastable crystalline phases are formed that can persist for long times. Eventually, these phases will transform to the stable phase. This behavior is usually interpreted in terms of Ostwald's rule of stages\(^41\). If the solute exhibits polymorphism, the various polymorphic forms will have a different solubility. In general, the solubility of the metastable forms will be higher than that of the stable form. To keep the reasoning simple, situations of compounds showing only two polymorphic forms are discussed. The generalization to cases of more than two polymorphic forms is straightforward. The thermodynamics of polymorphic systems is treated in detail and illustrated with various experimental examples by Burger and Ramberger\(^42,43\). Methods to determine the relative thermodynamic stability of polymorphs have been treated by Yu\(^44,45\). The effect of kinetics that favors the formation of metastable forms is discussed in terms of metastable zones by Threlfall\(^46\). In terms of the thermodynamics of solutions there is an important difference between monotropic and enantiotropic polymorphism. For a monotropic system there is only one stable form at all temperatures at a given pressure. In that case, in thermodynamic equilibrium, the metastable phase will always be dissolved and the stable form can be in equilibrium with the solution as described by equation (5). For an enantiotropic polymorphic system, however, there is a phase transition temperature below which one of the two polymorphic forms (say form I) is stable and the other one is metastable, while above that temperature form II is stable and form I is metastable. This implies that at the temperature $T_{trs}$ it holds that $\mu_{B1}^s(T_{trs}) = \mu_{BII}^s(T_{trs})$. Any polymorphic form has its own (metastable) S-L phase diagram. In case of an enantiotropic polymorphic compound $B$ the equilibrium phase diagram shows an additional feature as depicted in figure 10 as compared to figure 4. At the transition temperature, $T_{trs}$, indicated as $T_{trs}^\beta$ in figure 10 the solubility (liquidus) curves for the two polymorphs cross. In the latter figure the liquidus of both phase diagrams are only shown above the transition temperature.
Figure 10: Typical S-L phase diagram of a binary mixture well miscible in the liquid phase but immiscible in the solid phase, for an enantiotropic polymorphic system of the solute B. The dashed part of the liquidus of the solute represents the solubility curve described in the present paper. At $T_{trs}^B$ a solid-solid phase transition between the polymorphic forms I and II occurs.

Only at the transition temperature both polymorphic solid forms can be in equilibrium with the solution:

$$\mu_{BII}^I (T_{trs}) = \mu_{BII}^I (T_{trs}) = \mu_{BII}^I (T_{trs}) + RT \ln a_B^I (T_{trs}).$$ (40)

Note, that this equation is independent of the solvent used \textsuperscript{47,8} This implies that in thermodynamic equilibrium the solubility vs. temperature curves of different polymorphs always cross each other at the same transition temperature $T_{trs}$. In this light, situations where concomitant polymorphism\textsuperscript{48} is observed for temperatures that differ from the phase transition temperature $T_{trs}$, always represent non-equilibrium situations.

A further consequence of enantiotropic polymorphism is that for temperatures below the transition temperature the integral in equation (14) contains a contribution $\Delta_{trs} h_B^* (T_{trs}) / T_{trs}$ \textsuperscript{2} for each of the models discussed above. For example, for the case that the difference in molar heat

\textsuperscript{8} Here it is assumed that surface free energy contributions as a result of solvent-solid interactions can be neglected, which is, usually, reasonable for practical crystal sizes, even in the case of precipitation. For most molecular crystals the surface energy becomes comparable to the bulk energy for crystal diameters of the order of some 1-10 nm.
capacity is independent of the temperature between and the fusion temperature of the solid phase melting at the highest temperature, equation (26) has to be replaced by

$$\ln x_B = \sum_{\text{pol}} \frac{\Delta_{\text{pol}} \mu_B^* - \mu_B^*}{RT_{\text{pol}}} + \frac{\Delta_{\text{fus}} \mu_B^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] + \frac{\Delta_{\text{sol}} C_P}{R} \left[ \frac{T_{\text{fus}}}{T} - \ln \frac{T_{\text{fus}}}{T} - 1 \right] - \ln \gamma_B. \quad (41)$$

Although, strictly a non-equilibrium situation, the presence of the metastable polymorphic form in a solution, possibly concomitant with the stable polymorph, can, in many cases, persist for a long time; long enough to determine a solubility curve ($x$ vs. $T$) for both polymorphic forms. Such cases are found both for monotropic$^{49,50}$ and enantiotropic$^{51}$ systems. In principle, a metastable polymorph corresponds to a local minimum in the Gibbs free energy. The metastable solid phase cannot find kinetic pathways to transform to the stable form in the time allowed by the experiment. The solubility of both polymorphs can, therefore, be described by equation (7), be it with the appropriate parameters for the two polymorphs according to

$$\ln x_{\text{pol}} = \frac{\mu^*_{\text{pol}} - \mu_B^*}{RT} - \ln \gamma_B. \quad (42)$$

Note, that the term $\ln \gamma_B$, describing the non-ideality of the solution, is the same for both polymorphs at any temperature $T$; the same holds for the chemical potential of the pure liquid solute, $\mu_B^*$. This implies that the difference in solubility of the two polymorphs at a temperature offers valuable information of the difference in chemical potential of their solid phases

$$\mu^*_{\text{pol}} - \mu_B^* = RT \left[ \ln x_{\text{pol}} - \ln x_B \right]. \quad (43)$$

As this equation involves pure compounds the chemical potentials can be rewritten in terms of the differences in enthalpy and entropy of the two polymorphs according to

$$\Delta_{\text{pol}} h^* - \Delta_{\text{pol}} s^* = RT \Delta_{\text{pol}} \ln x_B. \quad (44)$$

Assuming a negligible temperature dependence for both $\Delta_{\text{pol}} h^*$ and $\Delta_{\text{pol}} s^*$, this equation can be used to determine $\Delta_{\text{pol}} h^*$ and $\Delta_{\text{pol}} s^*$ as the slope and intercept with the $y$-axis.
in a $\Delta \ln x_B$ vs. $1/RT$ plot for the temperatures considered. This was used, for example by Stoica et al. to determine the dissolution enthalpy and entropy of two polymorphic forms of a steroid$^{50}$. Moreover, equation (44) implies that the transition temperature for an enantiotropic system is determined by

$$T_{trs} = \frac{\Delta_{H\rightarrow S}^*}{\Delta_{H\rightarrow I}^*}$$

(45)

as for any equilibrium phase transition. This temperature, when neglecting the difference in heat capacity between the two polymorphs in the temperature range between the transition temperature $T_{trs}$ and $T_{fus}$, is given by

$$T_{trs} = \frac{\Delta_{fus}^* h_{Blu}^* - \Delta_{fus} h_{Bl}^*}{\Delta_{fus}^* h_{Blu}^* / T_{fus}^* - \Delta_{fus} h_{Bl}^* / T_{fus}^*}$$

(46)

Therefore the transition temperature can, besides from solubility curves, be estimated from DSC measurements as long as no solid-solid transition between the polymorphic forms occurs during such an experiment. This has been successfully applied to three enantiotropically related polymorphs of venlafaxine$^{52}$.

In case the transition can not be avoided and the transition temperature $T_{trs}$ and the corresponding enthalpy change $\Delta_{trs}^* h^*$ are available, e.g. from accurate DSC experiments, one can use this information to estimate the solubility ratio of the polymorphs using the method described by Mao et al.$^{53}$.

2.3.1 Pseudo polymorphs and solvates

The term pseudo polymorphism was originally used for crystal structures that can contain guest molecules in various amounts, e.g. solvent molecules, not influencing the structure of the host lattice. Solvates are crystals for which the crystal structure contains a stoichiometric amount of solvent molecules at well-defined crystallographic positions. In the case of water being the solvent the term hydrates is used. Solvates can therefore be considered as compounds of the form $A_p B_q$, where A represents the solvent and B the pure solute. In the field of polymorphism the term pseudo polymorphs is often used referring to solvates. Figure 11 shows a typical composition-temperature phase diagram of such a system for which, two solid phases are stable: a pure solid phase B and a solvate AB. As before, the solid phase of the solvent is not considered as it is out of the experimental temperature range as indicated by the dashed solubility
curves. The maxima in the solubility curves correspond to the fusion temperatures of the solid phases. Starting from these maxima the solubility curves can, again, be considered as freezing point depression curves as a result of the admixture of one of the other phases to the liquid phase, the curves ending in the eutectic points $x_c$ at the temperatures $T_{E,AB}^A$ and $T_{E,AB}^B$, respectively. Within the hatched areas the lever rule can be applied to determine the amounts of the two phases in equilibrium. In figure 11 the phase diagram limited to the composition interval $0 < x_B < 0.5$ can be interpreted as an S-L phase diagram comparable to that of figure 4 with B replaced by AB; a same comparison can be made for the interval $0.5 < x_B < 1$, A and B being replaced by AB and B, respectively. Concentrating on the composition interval $0.5 < x_B < 1$ the negative slope of the solubility curve bounding the $l + s_{AB}$ region should not be interpreted as a retrograde solubility in this case. In practice, more than one solvate or pseudo polymorphic form can be stable in various temperature and composition intervals, leading to as so many maxima and eutectic points in the phase diagram$^{54}$.

Figure 11: Typical S-L phase diagram of a binary mixture well miscible in the liquid phase but only stoichiometrically miscible in the solid phases, leading to a pure solid phase B and a solvate AB; in case of molecular crystals such a solvate is often called a pseudo polymorph.
2.4 Conclusion

Although many methods have been developed for a quantitative prediction of vapor liquid equilibrium (VLE) phase diagrams in the last century, much less attention has been given to solid liquid equilibria (SLE). SLE phase diagrams contain the essential information for determining the solubility of a compound in a solvent. Solubility has been studied in great detail at the beginning of the last century. The thermodynamic basis of solubility theory seems to have lost attention since then. In the last few decades solubility of especially organic molecules has regained interest as a result of the discovery of many new organic molecules in the field of pharmaceuticals. Not only the synthesis and purification of these compounds ask for suitable solvents and therefore solubility data, but also the frequent appearance of various polymorphic crystal forms of these compounds, often related to the solubility properties in different solvents, have led to an increasing demand for reliable solubility data.

In this paper the thermodynamic basis of solubility theory is reviewed and the link with binary phase diagrams is emphasized for that. Starting from the simplest solubility model, i.e. the ideal solubility case, models with increasing complexity are treated. For that a mean field approach is used. The resulting solubility curves as a function of temperature are presented for various values of the relevant thermodynamic parameters. Special situations, like liquid-liquid separation (oiling out) and retrograde solubility are highlighted. It is, for example, shown that the difference in heat capacity between an undercooled melt and the solid, a parameter which is almost always neglected, in the case of more complex molecules like many pharmaceuticals, can have a considerable effect on the solubility. Special attention is given to the solubility phase diagrams of monotropic and enantiotropic polymorphic forms as well as solvates (pseudo polymorphs). In passing a new method is introduced that allows to estimate the transition temperature of enantiotropically related polymorphs from melting temperatures and enthalpies of the polymorphs, which can be determined using standard calorimetric techniques.
2.5 Appendix A. The pure solute parameters

The general solubility equation (14) involves a pure solute parameter, $\Delta_{s \rightarrow l} \mu_B^*$, according to

$$\frac{\Delta_{s \rightarrow l} \mu_B^* (T)}{RT} = \frac{\mu_B^* (T) - \mu_B^* (T)}{RT} = \frac{1}{R} \int_{T}^{T_{fus}} \left( \frac{\Delta_{s \rightarrow l} h_B^* (T')}{(T')^2} \right) dT'. \quad (47)$$

Although, as mentioned before, $\Delta_{s \rightarrow l} h_B^*$ can be determined as a function of the temperature by measuring the heat of solidification of the supercooled liquid, various approximations have been made in the literature to come to an expression in terms of an experimentally more accessible parameter. Usually, such approximations are made for the molar heat capacity $c_p$, rather than for $\Delta_{s \rightarrow l} h_B^*$. Therefore, first equation (14) is rewritten in terms of the molar heat capacity $c_p$, using $h(T) = h(T_{fus}) + \int_{T_{fus}}^{T} c_p dT$ as

$$\ln x_B = \frac{\Delta_{fus} h_B^*}{R} \left[ \frac{1}{T_{fus}} - \frac{1}{T} \right] - \frac{1}{R} \int_{T_{fus}}^{T} \left( \frac{\Delta_{s \rightarrow l} c_p dT'}{(T')^2} \right) dT', \quad (48)$$

where $\Delta_{s \rightarrow l} c_p = c_p^l - c_p^s$; Equation (48) applies as long as the solute does not sublime before melting and there is no solid state phase transition between $T$ and $T_{fus}$. The term $\ln \gamma_B$ is set equal to zero as only the pure solute terms are considered in this appendix. In equation (48) the first term on the right-hand side describes the contribution to the solubility due to the jump in the enthalpy as a result of the fusion process at the fusion temperature; the second term accounts for the differences in enthalpy going from the temperature $T$ to $T_{fus}$. In figure 12 these contributions are illustrated.

The sharp peak in figure 12b and the corresponding jump in figure 12a mark the phase transformation and are an idealization of the more spread behavior found in calorimetric measurements. In practice, the transition from the solid to the liquid phase always starts
immediately at $T_{\text{fus}}$ during heating, while the liquid phase can, often, be undercooled below the fusion temperature. Therefore, the difference in heat capacity $\Delta_{s\rightarrow l}c_p$ cannot be determined above the fusion temperature, while for temperatures below $T_{\text{fus}}$ a non-zero value can be measured. The temperature dependence of $\Delta_{s\rightarrow l}c_p$ from $T$ to $T_{\text{fus}}$ is described by the second term on the right-hand side of equation (48). Although the temperature dependence of the heat capacity of the solid phase and the undercooled liquid phase can be far from linear it is to be expected that the difference of these, that is $\Delta_{s\rightarrow l}c_p$, is only moderately dependent on the temperature. Below, some of the approximations found in the literature for the temperature dependence of $\Delta_{s\rightarrow l}c_p$ are mentioned.

\[ \Delta_{s\rightarrow l}c_p(T) = c_p^s(T) - c_p^l(T) = \Delta c_p^0 + \Delta c_p^1(T - T_{\text{fus}}) \]  

Equation (23) describes the solubility curve when the difference in molar heat capacity is neglected between $T$ and $T_{\text{fus}}$, leading to a linear relation between $\ln x_B$ and $1/T$ in a classical solubility plot for ideal solutions.

Often an expansion of the difference in molar heat capacity for both phases, in powers of the temperature $T$, around $T_{\text{fus}}$ is made. Here, only a linear dependence on the temperature as suggested in figure 12b, according to

\[ \Delta_{s\rightarrow l}c_p(T) = c_p^s(T) - c_p^l(T) = \Delta c_p^0 + \Delta c_p^1(T - T_{\text{fus}}) \]  

\[ (49) \]
is considered. This leads to a quadratic temperature dependence of the difference in molar enthalpy according to

$$\Delta_{\text{sol}} h_B^* (T) = \Delta_{\text{fus}} h_B^* + \Delta c_p^0 (T - T_{\text{fus}}) + \frac{\Delta c_p}{2} (T - T_{\text{fus}})^2.$$  

(50)

Substituting equation (50) in equation (47) leads to the ideal solubility equation

$$\ln x_B = \frac{\Delta_{\text{fus}} h_B^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] + \frac{\Delta c_p^0}{R} \left[ \frac{T_{\text{fus}}}{T} - \ln \frac{T_{\text{fus}}}{T} - 1 \right] + \frac{\Delta c_p T_{\text{fus}}}{2RT} \left[ \frac{T}{T_{\text{fus}}} - \frac{T_{\text{fus}}}{T} + 2 \ln \frac{T_{\text{fus}}}{T} \right].$$  

(51)

The non-zero difference in enthalpy at the transition temperature $T_{\text{fus}}$ is covered by the first term on the right-hand side of the equation. In all these approximations the temperature is limited to $T \leq T_{\text{fus}}$ and it is assumed that the transition to the liquid phase is instantaneous, or in other words, the temperature is increased slowly enough to allow the solid to melt in the experimental time. For a discussion of the effects of too fast temperature runs in a DSC experiment the reader is referred to$^{55}$.

2.6 Appendix B. $C_p$-contribution: Venlafaxine as an example

To get an impression of the contribution of the difference in molar heat capacity of the undercooled liquid and the crystal, $\Delta_{\text{sol}} c_p (T)$, to the solubility expression (26) the heat capacities for venlafaxine$^{52}$ were measured using a Mettler Toledo DSC822$^e$. For that measurement 15 mg of venlafaxine was loaded to one of the calorimeter cups, the reference vessel left empty. The material was >99.9 % grade. The sample was heated at a rate of 10º min$^{-1}$ starting from room temperature up to a temperature well above the melting temperature ($T_{\text{fus}} = 348.1 K$), subsequently cooled down to a temperature at which the undercooled melt did not recrystallize yet. Starting from that temperature a second heating run was performed, again, up to a temperature well above the melting temperature. To determine the heat capacities accurately, the measurement was preceded by two runs using the same cups; one performed for two empty cups and a second one for which one of the vessels was loaded with Al$_2$O$_3$ as a reference sample. The final temperature dependence of the $c_p$-values of venlafaxine was determined using the equation
where \( HF \) is the measured heat flow as a function of the temperature and \( m \) is the mass of the sample. The resulting curves are shown in figure 13.

\[
C_{p,\text{sample}}(T) = \frac{HF_{\text{sample}} - HF_{\text{empty}}}{HF_{\text{Al}_2\text{O}_3} - HF_{\text{empty}}} \frac{m_{\text{Al}_2\text{O}_3}}{m_{\text{sample}}} C_{p,\text{Al}_2\text{O}_3}(T),
\]

(52)

*Figure 13: The measured \(-\)-curves of venlafaxine for a heating run (black trace) starting at room temperature up to a temperature well beyond the melting temperature \((T_{\text{fus}} = 348.1 \text{ K})\), followed by a cooling run down to a temperature at which recrystallization did not occur (not shown). The second heating run (red trace) for the undercooled melt was made again up to temperatures well beyond the melting temperature; the deviations at low temperatures are an artifact of the measurement.*

The deviations at low temperatures are an artifact due to the non-reliable data obtained with the device used at the start of any heating run. Undercooled venlafaxine can be cooled down to a temperature of approximately 310 K without solidification. The difference in heat capacity between solid venlafaxine and its undercooled liquid phase turns out to be almost independent of temperature over the entire range from 310 K up to \( T_{\text{fus}} \) and is determined to be \( \Delta_{s\rightarrow l}c_p(T) \approx \Delta c_p^0 = 145 \text{ J/mol K} \). Using this value together with \( T_{\text{fus}} = 348.1 \text{ K} \) and \( \Delta_{\text{fus}}h_B^* = 27.2 \text{ kJ/mol} \) in equation (26), the effect of \( \Delta_{s\rightarrow l}c_p(T) \) on the solubility can be calculated. In figure 14 the results are shown.
Figure 14: The solubility curves according to equation (26) for venlafaxine using $\Delta_{\text{fus}}f_{\text{H}}^{*} = 27.2 \text{ kJ/mol}$ and $T_{\text{fus}} = 348.1 \text{ K}$. The heavy black curve represents the ideal solubility case for which $\Delta c_{p}^{0} = 0$. The green line shows the results for venlafaxine for which $\Delta c_{p}^{0}$ was determined to be 145 J/mol K. For comparison the blue curve shows the case for $\Delta c_{p}^{0} = 200 \text{ J/mol K}$. The dashed black line indicates $T_{\text{fus}}$. Figure b) shows the corresponding van 't Hoff curves of a).

The figure shows that for the case of venlafaxine the solubility deviates considerably from the ideal solubility curve for which $\Delta c_{p}^{0} = 0$. Moreover, it shows that for somewhat larger values for $\Delta c_{p}^{0}$ retrograde solubility can occur within an accessible temperature range.

As a second example, for naphthalene the same measurement was performed, although its liquid phase could be undercooled only some ten degrees below its fusion temperature. In this case $\Delta_{s\rightarrow p}c_{p}(T)$ is relatively small and slightly increases with decreasing temperature. The estimated average value of $\Delta_{s\rightarrow p}c_{p}(T) \approx 20 \text{ J/molK}$ leads only to a very small deviation from the ideal solubility curve as can be seen in figure 15.
Figure 15: The solubility curves according to equation (26) for naphthalene using \( \Delta_{fus} h_B^\circ = 19.1 \text{ kJ/mol} \) and \( T_{fus} = 353.4 \text{ K} \). The heavy black curve represents the ideal solubility case for which \( \Delta c_p^0 = 0 \), the green line the case of naphthalene for which \( \Delta c_p^0 \approx 20 \text{ J/mol K} \). For comparison the blue curve shows the case for \( \Delta c_p^0 = 200 \text{ J/mol K} \). The dashed black line indicates \( T_{fus} \). Figure b) shows the corresponding van’t Hoff curves of a).

### 2.7 Appendix C. Liquid-Liquid separation

In the regular solution model discussed in section 2.4 figures 8a,b show a horizontal plateau in the solubility curves beyond a critical positive value of \( \Delta_{mix} h_B \). For the corresponding temperature the solubility curves have to be replaced by horizontal lines in the \((x,T)\) diagrams, resulting in the coexistence of an A-rich and a B-rich solution in thermodynamical equilibrium with the solid phase. The coexistence of two liquid phases is also known as Liquid-Liquid (L-L) separation. Here, the thermodynamics behind L-L separation will be reviewed shortly. In figure 16a,b the solubility curve according to equation (35) for two of the curves in figure 8b have been redrawn without the horizontal lines. According to equations (32) and (35) the solubility curve describes the dependence of the chemical potential of the solute in the liquid phase, \( \Delta_{mix}^\text{reg} \mu_B \), on the mole fraction \( x_B \) up to a constant. As a result of the minimum free energy in thermodynamic equilibrium, it holds that

\[
\left( \frac{\partial^2 G}{\partial n_B} \right)_{P,T,n_A} = \left( \frac{\partial \mu_B}{\partial n_B} \right)_{P,T,n_A} \geq 0
\]  

(53)
as has been demonstrated in detail by Landau and Lifshitz. The equality in equation (53) corresponds to a set of critical points, that define the borders of an unstable situation. Substituting eq. (32) one obtains for this equality condition

\[ T = \frac{2\Delta_{\text{mix}}^\text{reg} h}{R} x_B (1-x_B). \]  

(54)

The corresponding curves, known as spinodals, have been drawn as the green lines in the \((x,T)\) diagrams of figures 16a and b. Note, that these spinodals describe the behavior of the liquid phase. In case of the presence of also a solid phase B, the critical points intersect with the solubility curve at certain temperatures, exactly in the extrema of the solubility curve. In between these minimum and maximum values the slope of the solubility curves is negative, which therefore corresponds to a thermodynamically unstable situation.

The red curves in figures 16a and b correspond to the values in the \((x,T)\) diagram where \(\Delta_{\text{mix}}^\text{reg} \mu_B = 0\) or, in other words, the Gibbs free energy of mixing in the liquid phase is minimal. These curves are defined by

\[ T = \frac{\Delta_{\text{mix}}^\text{reg} h}{R} \frac{(2x_B-1)}{\ln(1-x_B) - \ln x_B}. \]  

(55)

and known as the binodals. For a liquid mixture without a solid phase present, the binodal defines the region in the \((x,T)\) diagram below which L-L separation takes place. When also a solid phase is present, in the present case the solid B, Gibbs’ phase rule restricts this region to a single temperature, which is defined by the intersection between the binodal and the solubility curve. The values of \(x_B\) of the two intersection points cannot be determined in an analytical form because it involves a transcendental equation. These have been determined numerically for the parameters of figures 16a and b, leading to the figures 16c and d where the vertical scale has been blown up to show the intersection points more clearly and the horizontal lines in the corrected blue solubility curve have been added.

Note, that within the L-L separation interval for the areas of the deviating solubility above and below the horizontal line are not equal, in contrast to the well-known example of the liquid-gas phase equilibrium of e.g. the van der Waals equation, where the so-called ‘Maxwell equal area rule holds’
Figure 16: Two of the solubility curves (blue lines) of figure 8b according to equation (35); $\Delta_{fus} h_B = 100$ kJ/mol and $T_{fus} = 570$ K. The red lines show the function of equation (55), the green lines that of equation (54) for a) $\Delta_{reg} h_B = 10$ kJ/mol and b) $\Delta_{reg} h_B = 20$ kJ/mol. Note, the different ranges for $T$ in a) and b. The graphs c) and d) show an enlarged vertical scale for a) and b), respectively; in these graphs the metastable solubility lines are indicated as dotted black lines.

The binodal and spinodal in the present model are symmetrical in $x_A$ and $x_B$. The top of both at $x_B = 1/2$ is given by

$$T = \frac{\Delta_{reg} h}{2R}$$  \hspace{1cm} (56)

Substituting this value in the solubility equation (35) one finds for the critical value of $\Delta_{mix} h$ for L-L separation...
\[ \Delta_{\text{mix}}^{\text{reg}} h = \frac{4\Delta_{\text{fus}}^* h_B^*}{2 \ln 2 - 1 + 2 \left( \frac{\Delta_{\text{fus}}^* h_B^*}{RT_{\text{fus}}} \right)} \]  

(57)

which for the parameters of figure 16 yields \( \Delta_{\text{mix}}^{\text{reg}} h = 9.392 \) kJ/mol, which is slightly smaller than the middle blue curve in the figure.

In section 2.5 an additional excess entropy was introduced leading to a so-called quasi regular solution model in the meanfield approximation. For this model the binodal and spinodal are still symmetrical in \( x_A \) and \( x_B \). The top of both at \( x_B = 1/2 \) is now given by

\[ T = \frac{\Delta_{\text{mix}}^{\text{reg}} h}{\Delta_{\text{mix}}^{\text{qu-reg}} s + 2R}, \]

(58)

leading to a critical value of \( \Delta_{\text{mix}}^{\text{reg}} h \) for L-L separation

\[ \Delta_{\text{mix}}^{\text{reg}} h = \frac{4\Delta_{\text{fus}}^* h_B^* \left( 1 + 2\Delta_{\text{mix}}^{\text{qu-reg}} s / R \right)}{2 \ln 2 - 1 + 2 \left( \frac{\Delta_{\text{fus}}^* h_B^*}{RT_{\text{fus}}} \right)}. \]

(59)

For the parameters of figure 9 this leads to \( \Delta_{\text{mix}}^{\text{reg}} h = 15.040 \) kJ/mol for \( \Delta_{\text{mix}}^{\text{qu-reg}} s = 10 \) J/mol K. This explains the absence of a plateau, indicating L-L separation, in figure 9a for \( \Delta_{\text{mix}}^{\text{reg}} h = 10 \) kJ/mol and \( \Delta_{\text{mix}}^{\text{qu-reg}} s = 10 \) J/mol K.

Summarizing, only at the temperature where the solubility curve intersects the binodal two liquid phases with mole fractions corresponding to the intersection points are in thermodynamic equilibrium with the solid phase of the solute B. For temperatures and mole fractions above the solubility curve but below the binodal (red line) the liquid also separates in two liquid phases, with no solid phase. For temperatures and mole fractions above the binodal only a single liquid phase is present.

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Chapter 3
THE SOLUBILITY BEHAVIOUR AND THERMODYNAMIC RELATIONS OF THE THREE FORMS OF VENLAFAXINE FREE BASE

Abstract

The polymorphic and solubility behavior of the active pharmaceutical ingredient Venlafaxine free base, which is used as an antidepressant, is studied. Using Differential Scanning Calorimetry and slurry experiments, an enantiotropic relation between the three forms was found. Transition temperatures were determined using solubility data and compared with calculated transition temperatures based on the melting enthalpies and temperatures of the different forms. The solubility of Venlafaxine in heptane, toluene and methanol shows a large deviation from ideal behavior. The deviations are to a large extent determined by the temperature dependence of the difference in fusion enthalpy of the undercooled melt and the solid.
3.1 Introduction

Polymorphism is a major concern for the pharmaceutical industry during the development of a new drug\textsuperscript{1,2}. Polymorphism is the ability of substances to crystallize in two or more crystalline phases. Each of these crystal structures, or modifications, has the same chemical composition, but differs in the arrangement and/or conformation of the molecules in the crystal lattice. As a result, polymorphic forms have different physical properties, e.g. melting point, solubility, dissolution rate, habit and stability. These different physical properties may affect the therapeutic efficacy, toxicity, bio-availability, pharmaceutical processing and stability of the drug product. An important part of the investigation of a new form of a pharmaceutical product is the comprehensive study for polymorphs. Firstly, because it may prevent patenting problems\textsuperscript{3} and secondly, it is recommended by regulatory bodies\textsuperscript{4}.

Two distinct types of polymorphic systems are known; monotropic and enantiotropic systems. When the free energy curves of the stable polymorph and any other observed polymorph do not cross as a function of temperature, and consequently only one polymorph is stable below the melting point, one deals with a monotropic system. When the free energy curves as a function of temperature of two polymorphs do cross below the melting point, different polymorphs are stable below and above the transition point, and one speaks of an enantiotropic system. Knowing the number of phases and their thermodynamic relationships in the context of crystallization is a basic pre-requisite in understanding the phase diagram of a polymorphic system.

During crystallization it is possible that a less stable form crystallizes out first, which can be transformed to a more stable one later. This is known as Ostwald’s rule of stages\textsuperscript{5}. The formation of metastable polymorphic crystals is a kinetic effect, which is usually attributed to the nucleation stage\textsuperscript{6,7}. Nevertheless, examples are known, for which the metastable phase is formed heterogeneously on the stable phase crystals either in a random orientation\textsuperscript{8,9} or epitaxially\textsuperscript{10,11}.

Interconversion of polymorphs can be accomplished via solvent or gas phase mediated transformation or directly via a solid-solid phase transformation. Depending on the kinetic barriers, the transformation time can range from instantaneous to infinitely long. Determination of the precise polymorph transition temperature for an enantiotropically related system is often a laborious task. For such a system the solubility of two polymorphs is, however, equal at the transition point. Therefore solubility data can be used to determine the transition temperature and the stability region of the polymorphs. Assuming that the solution shows more or less ideal
mixing behaviour for the solute and solvent, solubility data will show up as nearly linear curves in a van ‘t Hoff plot. If the polymorphic transition rate is low, it is possible to determine the solubility of both polymorphs even in the region in which the polymorphs are metastable.

In the following we report a study of the phase behaviour of the free base of the drug Venlafaxine. The polymorphic phase diagram as well as the thermodynamic relations between the three forms were investigated using X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), slurry experiments and solubility measurements. The solubility of Venlafaxine was studied in different solvents, with the emphasis on deviations of the solubility with respect to the van ‘t Hoff equation for ideal behaviour. The deviations turn out to be such that the assignment of the stability regions of polymorph I and II of Venlafaxine, on the basis of solubility data, would lead to a contradiction. This is due to a concave form of the solubility curves, a shape that was recently described in detail in terms of a thermodynamic treatment of solubility. In that paper an equation to calculate the transition temperature of two enantiotropically related polymorphs was published. For this equation only the melting point and the melting enthalpy, which are relatively easy to determine using DSC, are necessary. The resulting transition temperatures for Venlafaxine free base are compared with those derived from the solubility data and the shape of the curves is interpreted in terms of the mixing properties in the solution. The concave shape is explained by a non-negligible difference in the temperature dependence of the enthalpy of fusion.

3.2 Venlafaxine

Venlafaxine is the common name for the compound 1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol. The molecular structure is given in figure 1.

![Figure 1: The molecular structure of Venlafaxine.](image)

The product is marketed as the hydrochloric acid salt. It is used for treating depression and thymoanaleptic and anxiolytic disorders. Especially because Venlafaxine hydrochloride is relatively aggressive towards handling equipment and it is irritating to the skin, it would be
beneficial to have other forms of Venlafaxine at one’s disposal. The free base is one of the promising candidates.

Until a few years ago, little was reported regarding Venlafaxine free base, the information being restricted to patents\textsuperscript{14,15}. In these disclosures Venlafaxine was never precipitated as a free base, but obtained as an oily/solid residue in the synthesis, after evaporation of the solvent. This procedure generally does not lead to a useful pharmaceutical solid form, as impurities and the solvent are likely to be present. Later, Venlafaxine free base was isolated as a solid via evaporation of the solvent and the resulting residue was recrystallized from hexane or heptane\textsuperscript{16}. The fact that it can be isolated in multiple forms was recently described\textsuperscript{17}. According to this publication, two polymorphs, A and B, of the free base of Venlafaxine exist. The crystal structures were not known and the thermodynamic behaviour was only poorly understood. Polymorph A was expected to be the stable form at room temperature.

Very recently it was reported that three forms of Venlafaxine free base exist and their crystal structures were determined using single crystal X-ray diffraction\textsuperscript{18}. This showed that in case of polymorphs I and II one deals with racemic compounds; form I is composed of alternating layers of R and S enantiomers, in case of form II the crystal is composed of alternating bi-layers of R and S enantiomers. Form III, a racemic conglomerate, is composed of a stacking of thick layers of R and S enantiomers, in other words, form III crystallizes as an epitaxial conglomerate. We will use the same labels as in ref. [18].

3.3 Solubility theory

In the following we summarize some of the theoretical considerations concerning solubility of (molecular) crystals\textsuperscript{13} that are relevant for the present study. The solubility curve of crystals, as a function of the temperature $T$, in case of an ideal mixing behaviour of the solute and the solvent and assuming that the fusion enthalpy of the pure solute, $\Delta_{\text{fus}} h^*$, is independent of the temperature follows from:

$$
\ln x_{\text{ideal}} = \frac{\Delta_{\text{fus}} h^*}{R} \left( \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right),
$$

where $x$ is the mole fraction of the solute in the solution. If $\ln x$ is plotted versus $\frac{1}{T}$ this leads to a linear solubility curve, often referred to as a van't Hoff curve. In practice, however, there will
be a temperature dependence of $\Delta_{\text{fus}} h^*$, which results, even for an ideal mixing in solution, in a deviation of the linear curve. Figure 2 presents an overview of the different shapes solubility curves can adopt. When $\Delta_{\text{fus}} h^*(T)$ is expressed in terms of a linear temperature dependence of the molar heat capacity according to:

$$c_p^T = c_p^T(T) - c_p^T = \Delta c_p^0 - \Delta c_p^T(T - T_{\text{fus}}),$$ (2)

where the superscript $^l$ denotes the (undercooled) melt and superscript $^s$ the solid phase, the solubility becomes:

$$\ln x^T = \frac{\Delta_{\text{fus}} h^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] + \frac{\Delta c_p^0}{R} \left[ T_{\text{fus}} - \ln \frac{T_{\text{fus}}}{T} - 1 \right] + \frac{\Delta c_p^T}{2R} \left[ \frac{T_{\text{fus}}}{T} - \frac{T_{\text{fus}}}{T} + 2 \ln \frac{T_{\text{fus}}}{T} \right].$$ (3)

If $\Delta c_p(T)$ is positive, the solubility is larger than the ideal solubility, if $\Delta c_p(T)$ is negative the solubility is lower. As a result, the solubility curve changes from a straight line for the ideal case, to a non linear curve; concave in case $\Delta c_p(T)$ is positive and convex in case $\Delta c_p(T)$ is negative. Note that this deviation is independent of the solvent used.

Deviations from ideal behaviour are usually expressed in terms of excess parameters, $\Delta_{\text{mix}}^E H$ and $\Delta_{\text{mix}}^E S$. In case of a regular solution\cite{19}, for which $\Delta_{\text{mix}}^E S = \Delta_{\text{mix}}^\text{reg} S = 0$, and $\Delta_{\text{mix}}^E H = n x (1-x) \Delta_{\text{mix}}^\text{reg} h$, the solubility is:

$$\ln x^\text{reg} = \frac{\Delta_{\text{fus}} h^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] - \frac{\Delta_{\text{mix}}^\text{reg} h}{R} \left[ (1-x^\text{reg})^2 \right].$$ (4)

If the regular solution model is extended, to include an excess mixing entropy, $\Delta_{\text{mix}}^E S = \Delta_{\text{mix}}^\text{qu-reg} S$, a quasi-regular solution model results for which the solubility is given by:

$$\ln x^\text{qu-reg} = \frac{\Delta_{\text{fus}} h^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] - \left( \frac{\Delta_{\text{mix}}^\text{reg} h - T \Delta_{\text{mix}}^\text{qu-reg} S}{R} \right) \left[ (1-x^\text{qu-reg})^2 \right].$$ (5)
The parameters $\Delta_{\text{mix}}^h h$ and $\Delta_{\text{mix}}^{\text{qu-reg}} s$ are assumed to be independent of the temperature in the range of interest. An important conclusion from figure 2 is that for a negligible $\Delta c_p$ value the solubility curves adopt a linear behaviour well below the melting temperature of the solute, as long as $\Delta_{\text{mix}}^h h$ and $\Delta_{\text{mix}}^s s$ are independent of the temperature, whereas a non-negligible (positive) value of $\Delta c_p$ leads to a concave shaped curve. If all parameters are included: $\Delta c_p (T), \Delta_{\text{mix}}^E H$ and $\Delta_{\text{mix}}^E S$, the solubility equation becomes:

$$
\ln x^{all} = \frac{\Delta_{\text{fus}}^h h^*}{R} \left[ \frac{1}{T_{\text{fus}}} - \frac{1}{T} \right] + \frac{\Delta c_p}{R} \left[ \frac{T_{\text{fus}}}{T} - \ln \frac{T_{\text{fus}}}{T} - 1 \right] + \frac{\Delta c_p T_{\text{fus}}}{2R} \left[ \frac{T}{T_{\text{fus}}} - \frac{T_{\text{fus}}}{T} + 2 \ln \frac{T_{\text{fus}}}{T} \right] - 
$$

$$
\left( \frac{\Delta_{\text{mix}}^h h - T \Delta_{\text{mix}}^{\text{qu-reg}} s}{R} \right) \left[ \left( 1 - \frac{x}{T} \right)^2 \right].
$$

(6)

In case of an enantiotropic polymorphic system the transition temperature, $T_{\text{trs}}$, is given by the following equation:

$$
T_{\text{trs}} = \frac{\Delta_{\text{trs}}^h h^*}{\Delta_{\text{trs}}^s s^*},
$$

(7)

where $\Delta_{\text{trs}}^h h^*$ and $\Delta_{\text{trs}}^s s^*$ are the enthalpy and the entropy change respectively, for the transition between the polymorphic forms at the transition temperature. Assuming ideal behaviour, according to equation (2), this temperature can be approximated by the following equation\textsuperscript{13}:

$$
T_{\text{trs}} = \frac{\Delta_{\text{fus}}^h h^*_\alpha - \Delta_{\text{fus}}^h h^*_\beta}{T_{\text{fus},\alpha} - T_{\text{fus},\beta}},
$$

(8)

where $\Delta_{\text{fus}}^h h^*_\alpha$ and $\Delta_{\text{fus}}^h h^*_\beta$ are the melting enthalpy of forms $\alpha$ and $\beta$, respectively, and $T_{\text{fus},\alpha}$ and $T_{\text{fus},\beta}$ are the respective absolute fusion temperatures.
The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base

Figure 2: Overview of the shapes solubility curves can adopt when various thermodynamic parameters are included in the calculations. The black curve represents the ideal case, equation (1); the green curve represents the case for which a temperature dependence of $\Delta_{fus} h^°(T)$ is included, equation (3); the blue curve represents the case for which only $\Delta_{reg}^m h$ is included, equation (4); the yellow curve represents the case for which both $\Delta_{mix} h$ and $\Delta_{s-mix} h$ are included, equation (5) and the red curve represents the case for which all parameters are included; equation (6). The following parameters are used for the calculations: $T_{fus} = 373 K$, $\Delta_{fus} h = 30 kJ/mol$, $\Delta_{c_p} = 150 J/molK$, $\Delta_{s-c_p} = 0$, $\Delta_{mix} h = 5 kJ/mol$ and $\Delta_{s-mix} h = -5 J/molK$.

3.4 Experimental Procedures

Venlafaxine with a purity of 99.9%, according to High Pressure Liquid Chromatography (HPLC), was supplied by Synthon BV. The solvents used (pa) were purchased from Aldrich and were used without further purification.

All the solubility experiments were started using polymorph I. Crystals were obtained by dissolving Venlafaxine in heptane at reflux temperature, crystallizing the different polymorphs by cooling the clear solution to the desired temperature, and isolating the formed crystals at that temperature.

For the determination of the solubility two different techniques were used. The most accurate, but time consuming, approach is an in situ method. For that a closed thermostated glass
cell was filled with a known amount of solute and solvent. The growth and dissolution of the crystals was observed using an optical transmission microscope (Zeiss Axioplan 2). By varying the temperature the saturation temperature for different concentrations was determined; after nucleation at low temperatures all the crystals were dissolved by increasing the temperature except for one very small one, in order to minimize the effect on the solute concentration. This crystal was subsequently grown and etched a few times to determine the saturation temperature of the solution. Besides being a time consuming method a disadvantage of the method is that it is difficult to determine the polymorphic form of the crystal studied.

The fast, but less accurate, method is the saturation shaken flask method. For that, a slurry of a given polymorph and a saturated solution was stirred for several days at a well defined temperature, allowing the appropriate polymorph to be formed. The residue was filtered off and the polymorph obtained was determined using X-ray powder diffraction. A certain amount of the clear solution was weighed and the solvent was evaporated using a rotary evaporator. From the weight of the residue and the weight of the solution the amount of solvent and solute was determined.

Differential scanning calorimetry was performed using a Mettler Toledo DSC 822e. Nitrogen was used as an inert purge gas. The samples were analyzed using 40 μl aluminium pans with a pierced lid. The heating rate varied between 0.1 and 10 °C/min. Further calorimetric measurements were performed using a Setaram C-80 micro calorimeter. In this case a heating rate of 0.01 or 0.001 °C/min was used. The heat capacity of polymorphs I, II and III was determined using a Mettler DSC 822e apparatus. The thermograms were recorded using 40 μl aluminium pans with a heating rate of 10 °C/min. Sapphire was used as standard.

X-ray powder diffraction patterns were recorded using a Bruker Vario-1 diffractometer in a Bragg-Brentano geometry. The patterns were collected in the range from 2 to 35° 2θ using a VÁNETC detector. Samples were measured in reflection mode using a silicon zero background wafer or a sampleholder with a silicon cavity. Cu Kα-2 radiation was used at a voltage of 40 kV and a current of 40 mA.

3.5 Results and Discussion

3.5.1 X-ray powder diffraction.

Figure 3 shows the X-ray powder diffractograms of the three forms of Venlafaxine. Comparing these with the diffractograms given in the patent, ref [18], of the forms called A and
B in that reference, we notice that polymorph I is identical to polymorph A. The XRPD pattern of form III resembles the pattern of form B, although extra peaks are visible in the pattern of form B. After careful comparison of the pattern of form B with the patterns of forms II and III, we conclude that form B is a mixture of forms II and III.

![XRPD patterns of forms I, II and III of Venlafaxine free base.](image)

**Figure 3: XRPD patterns of forms I, II and III of Venlafaxine free base.**

3.5.2 Slurry experiments.

To obtain a first impression of the mutual stability of the three forms at different temperatures, slurry experiments were performed, starting with different forms. For the results of these experiments see table 1. Under the right conditions, in a slurry, all the different forms can be converted into each other. It can be concluded from these slurry experiments that an enantiotropic relation exists between all the three forms. The stability regions are as follows; below ~20 °C form I is stable, above ~55 °C form III is stable. In a region somewhere between these temperatures, form II is stable.
Table 1: Results of the slurry experiments.

<table>
<thead>
<tr>
<th>starting polymorph</th>
<th>isolated polymorph</th>
<th>conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>II</td>
<td>stirring at 42°C, for 7 days</td>
</tr>
<tr>
<td>II</td>
<td>I</td>
<td>stirring at 20°C, for 7 days</td>
</tr>
<tr>
<td>I</td>
<td>III</td>
<td>stirring at 55°C, for 7 days</td>
</tr>
<tr>
<td>III</td>
<td>II</td>
<td>stirring at 42°C, for 7 days</td>
</tr>
<tr>
<td>III</td>
<td>I</td>
<td>stirring at 20°C, for 7 days</td>
</tr>
<tr>
<td>II</td>
<td>III</td>
<td>stirring at 55°C, for 7 days</td>
</tr>
</tbody>
</table>

In section 5.4 we will compare these calculated phase transitions temperatures with more precise transition temperatures determined using solubility data.

3.5.3 Thermographic analysis

A DSC study was performed to determine the thermodynamic behaviour of the polymorphs. A DSC thermogram of Venlafaxine free base, polymorph I, with the usual heating rate of 10°C/min showed one broad melting peak at 76 °C. There was no sign of a solid-solid transformation. Because solid-solid transformations can be difficult, due to kinetic barriers, the DSC measurements of polymorph I, were therefore repeated at lower heating rates. For the resulting thermograms see figure 4. When the heating rate is lowered from 10 to 1°C/min, two endothermic peaks are visible in the DSC thermogram, of which the first can be attributed to the melting of polymorph I, and the second one is the result of melting of another polymorph. In between these two endotherms, a small exothermic recrystallization peak is visible. Lowering the heating rate to 0.1 °C/min yields a small endothermic peak and a small exothermic peak, separated by only 0.3 degrees; the melting and the recrystallization processes now take place almost simultaneously. The complete sample is thus converted to another polymorph, which melts at ~ 78.7°C, with a melting enthalpy comparable to that of the fast run; for data see table 2.
The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base

Figure 4: DSC thermograms of polymorph I heated at 10, 1, 0.1 and 0.01 °C/min; for the sake of clarity the vertical axis of the traces have been enlarged; by a factor of 20 for the 0.01 °C/min trace, by a factor of 10 for the 0.1 °C/min trace and by a factor of 5 for the 1 °C/min trace.

Table 2: Thermal data obtained from the DSC scans of figure 4, the most right column states the sum of the thermic events.

<table>
<thead>
<tr>
<th>heating rate °C/min</th>
<th>T onset first peak °C</th>
<th>Δ H (J/g)</th>
<th>T onset second peak °C</th>
<th>Δ H (J/g)</th>
<th>T onset melting peak °C</th>
<th>Δ fusH (J/g)</th>
<th>Total Δ H (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.0</td>
<td>100.9</td>
<td>100.9</td>
</tr>
<tr>
<td>1</td>
<td>74.5</td>
<td>87.9</td>
<td>77.5</td>
<td>-0.5</td>
<td>78.3</td>
<td>14.6</td>
<td>102</td>
</tr>
<tr>
<td>0.1</td>
<td>74.4</td>
<td>7.4</td>
<td>74.7</td>
<td>-1.9</td>
<td>78.1</td>
<td>93.2</td>
<td>98.7</td>
</tr>
</tbody>
</table>

An experiment with an even lower heating rate of 0.01°C/min, performed with a C-80 Setaram calorimeter, resulted in a considerable shift of the first endotherm to 60°C with a heat of 2.9 J/g. The sign and the magnitude of the first endotherm are in accordance with the value of a solid-solid phase transition to an enantiotropically related polymorph. To verify this assumption, a sample was heated to 70°C in the C-80 Setaram calorimeter, as described above
(0.01 °C/min), and cooled to room temperature, after which an XRPD was recorded of the sample. The XRPD pattern showed that form III was isolated.

A DSC heating run of polymorph II with a heating rate of 10 °C/min or 1 °C/min showed only a melting endotherm at 78 °C. For an overview of the thermograms of polymorph II see figure 5.

Figure 5: DSC thermograms of polymorph II heated with different heating rates, 10, 1, 0.1 °C/min and 0.01 °C/min; for the sake of clarity the vertical axis of the traces have been enlarged; by a factor of 20 for the 0.01 °C/min trace, by a factor of 10 for the 0.1 °C/min trace and by a factor of 5 for the 1 °C/min trace.

Lowering the heating rate to 0.1 °C/min did not show any further peaks, only the onset of the melting peak was shifted to 77° C. Further lowering of the heating rate to 0.01 °C, using the C-80 Setaram calorimeter, did not lead to a well-resolved recrystallization exotherm and no solid-solid transformation is visible. The XRPD pattern of a sample of polymorph II, heated to 75° C with 0.01° C/min and then cooled to room temperature, was identical with the pattern of
the starting polymorph II, showing that no transformation had taken place. Starting with form III, the thermograms show only the melting peak of that polymorph, whatever heating rate was used.

The results given above show that a precise determination of the melting points and the melting enthalpies is difficult, especially for polymorph I. The result is an overestimation of the melting temperatures of polymorph I when a high heating rate is used. If a lower heating rate is used, the endothermic melting peak overlaps the exothermic crystallization peak of form III and as a result of that, it is not possible to determine the melting enthalpy accurately. To find a compromise with a reasonable accuracy, a heating rate of 3°C/min for polymorph I was used which resulted in the formation of only small amounts of form III. Likewise, overlapping peaks make it difficult to determine the melting enthalpy of polymorph II. The melting points and the melting enthalpies of all three polymorphs, thus obtained, are summarized in table 3.

Table 3: melting temperatures, melting enthalpies and heat capacity data of forms I, II and III of Venlafaxine. Δcp is determined from measurements between T_{fus} and 40°C (section 5.4).

<table>
<thead>
<tr>
<th></th>
<th>T_{fus} (°C)</th>
<th>Δ_{fus}H (J/g)</th>
<th>Δc_{p}^{0} (J/molK)</th>
<th>Δc_{p}^{1} (J/molK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorph I</td>
<td>74.9</td>
<td>98.1</td>
<td>136.4</td>
<td>-0.3355</td>
</tr>
<tr>
<td>Polymorph II</td>
<td>76.5</td>
<td>95.3</td>
<td>100.1</td>
<td>-0.3283</td>
</tr>
<tr>
<td>Form III</td>
<td>78.1</td>
<td>87.9</td>
<td>96.0</td>
<td>-0.109</td>
</tr>
</tbody>
</table>

Table 3 shows that the melting point of polymorph II is higher than that of polymorph I. Using the Burger and Ramberger thermodynamic “rules” for polymorphs²², this suggests that polymorph II has a lower free energy than polymorph I, at least near the melting point. The same reasoning is valid for forms III and II. These conclusions are in accordance with the results found in the slurry experiments.

3.5.4 Solubility experiments

3.5.4.1 Solubility of Venlafaxine in Heptane

Heptane, is used in the large scale purification of Venlafaxine free base, and was therefore included in this study. The polymorphic transition rate in this solvent is too high to determine the solubility of the polymorphs in their metastable regions. The solubilities of all three forms were determined using the saturation shaken flask method and are presented in figure 6.
Figure 6: van ‘t Hoff solubility plots of polymorphs I (open symbols) and II (closed symbols) in heptane, determined using the saturation shaken flask method, with extrapolated dashed trend lines.

A transition temperature between polymorphs I and II, of 35.7 °C and between form II and III at 51 °C was determined by extrapolating the trendlines, assuming a linear relation. Surprisingly, the extrapolated solubility line of polymorph I, which is stable at low temperatures according to the slurry experiments, shows that polymorph I has the lowest solubility above the transition temperature. For polymorph II a similarly surprising result is found for temperatures below that temperature. To investigate the cause of this anomalous behaviour, the solubility of Venlafaxine was determined over a wider temperature range, from -76 to 60 °C and compared with the van ‘t Hoff solubility curve for ideal solutions. To test the reliability of the saturation shaken flask method, solubility data for polymorph II were also determined using the in situ method. The combined solubility data, are presented in figure 7, in a van ‘t Hoff plot. In this figure the solubility of VFX is presented together with the various solubility curves calculated using the measured parameters presented in table 3, for polymorph I (solid curves) and II (dashed curves). From the determined solubility of Venlafaxine free base it is obvious that except for temperatures just below the melting point of the solute, the data deviate considerably from the ideal solubility behavior.
The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base

Figure 7: Solubility data of the three forms of Venlafaxine in heptane, determined down to 197 K; diamond symbols: data determined using saturation shaken flask method; square symbols: data determined using the in situ method; the solid and dashed black curves, represent the ideal solubility curves for polymorph I and II respectively (equation (1)), the red curves represent the solubility curves for polymorph I and II respectively for which a temperature dependence of $\Delta_{\text{fus}} h^* (T)$ is included using the data of table 3; the blue curves represent a fit for which all parameters are included, combining equation (6), the data of table 3 and $\Delta_{\text{mic}} h = 26.5 \text{ kJ/mol}$ and $\Delta_{\text{mic}} s = 72 \text{ J/molK}$.

The observed lower than ideal solubility could be accounted for by less favourite interactions between solvent and solute molecules. If the solubility data would be fitted using equation (5), which includes mixing terms only, the agreement would still be not very good, because as is shown in figure 2, the mixing terms in the equation can not account for the observed concave shaped curve in the solubility at lower temperatures. The concave shape suggests an effect of a temperature dependent $\Delta_{\text{fus}} h^*$. To determine $\Delta_{\text{fus}} h^*$, the difference of the molar heat capacity of the undercooled liquid and the crystal was measured. For most compounds it is rather difficult to determine this difference, because the undercooled liquid can usually be maintained only in a limited temperature range below the melting point. For
Venlafaxine, determination of $\Delta C_p$ turned out to be quite well possible down to 40 °C for the undercooled liquid. For that, a sample of Venlafaxine was heated to a temperature 10 °C above the melting point, cooled to 40 °C (without crystallization of the liquid) and the undercooled liquid was again heated to 10 °C above its melting point. As an example the resulting curves for polymorph I are shown in figure 8.

![Figure 8: $C_p$-curves of Venlafaxine. Heating run for the crystal (black curve), and heating run for the undercooled liquid (red curve). Deviations in the curves at low temperatures are artefacts of the measurement.](image)

Fitting the $C_p$-data to equation (2) resulted in parameters $\Delta c_p^0$ and $\Delta c_p^1$ for the three forms as presented in table 3. Including a temperature dependence for $\Delta_f^* h^*$ in the solubility equation, results in the red curves in figure 7. Combining the effect of the measured temperature dependence of $\Delta_f^* h^*$, an enthalpy of mixing and an excess entropy, as parameters to fit the solubility data, and using equation (6), the agreement between the calculated and the measured solubility is reasonable (blue curves in figure 7). The fit parameters used for these curves are presented in table 4.
Table 4: Fit parameters found for the different solvents using equation (6) and data of table 3.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\Delta_{mix} h$ (kJ/mol)</th>
<th>$\Delta_{mix} s$ (J/molK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>heptane</td>
<td>26.5</td>
<td>72</td>
</tr>
<tr>
<td>toluene</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>methanol</td>
<td>1.6</td>
<td>10</td>
</tr>
</tbody>
</table>

Note that the mixing parameters in the solution are independent of the form I, II or III. The agreement between the calculated and the observed solubility is especially good from 40 up to $\sim$ 60 °C. Below 40 °C the observed solubility shows a higher solubility than calculated. The positive deviation of the solubility could be the result of a more complex temperature dependence of $\Delta C_p$ at lower temperatures. After all it was not possible to measure $\Delta C_p$ below 40 °C. Although the concave shape of the solubility curves is described rather well by the temperature dependence of the heat of fusion difference, the order of the curves for polymorph I and II is swapped on including this difference.

3.5.4.2 Solubility of Venlafaxine in Toluene

To investigate the effect of different solvents on the solubility behaviour of Venlafaxine free base, the solubility was also determined in toluene, a solvent with physical parameters comparable to heptane; small difference in dipole moment and boiling point. The solubility of venlafaxine free base was determined in toluene using the saturation shaken flask method, in the temperature range of -25 °C to 60 °C, see figure 9. In case of toluene it was very difficult to measure the solubility at temperatures below -25 °C, because the solution became so viscous, that it was almost impossible to filter the solution keeping it at the right temperature. Like for heptane, the solubility behaviour of venlafaxine in toluene deviates considerably from ideal behaviour, but this time the deviation is positive compared to the ideal linear van ‘t Hoff curve. The shape of the solubility curve is still concave, as expected for a temperature dependent contribution of $\Delta_{mix} h^*$ to the solubility. When equation (6) is used as a model to fit the solubility data, the agreement is very good. The fit parameters found, using the data from table 3, are presented in table 4. Over the complete experimental temperature range the measured solubility follows the calculated curve very well. As for heptane, however, the order of the solubility curves of polymorph I and II is swapped.
3.5.4.3 Solubility of Venlafaxine in methanol

To study the behaviour of Venlafaxine free base in a polar solvent, the solubility was measured in methanol. The results are presented in figure 10. The data show that the solubility behaviour is not ideal, but the deviation from ideal behaviour is not large, besides the data point at low temperature, which deviates considerably, like in the case. Using equation (6), to fit the data, the results are in a reasonable agreement between the observed and the calculated solubility. The fit parameters using the data from table 3, are presented in table 4.
3.5.5 Discussion

From the comparison between the measured and the calculated solubility of Venlafaxine it is clear that the temperature dependence of $\Delta_{fus} h^\ast$ has a large effect on the solubility. That this effect is independent of the solvent used, is clear from the fact that the resulting concave shape of the solubility curve was observed both for polar and apolar solvents. For Venlafaxine it was possible to measure $\Delta c_p$ down to 35 degrees undercooling. For polymorph I the measurement of $\Delta c_p$ is less accurate because during the first heating cycle part of the solid can transform to polymorph II via a melt-recrystallization process. The same effect makes it difficult to determine the precise melting temperature and melting enthalpy for polymorph I. Together with the instrumental errors, these effects might account for the observed swap of the position of the calculated ideal solubility curves of polymorph I and II on including the effect of $\Delta c_p$. 

Figure 10: Solubility data of the three forms of Venlafaxine in methanol, open diamond symbols, data determined using the saturation shaken flask method; the solid black curve representst the ideal solubility curves for polymorph I the red curve represent the solubility curve for polymorph I for which a temperature dependence of $\Delta_{fus} h^\ast(T)$ is included; and the blue curves represent the case for which all parameters are included. For the calculations data from table 2 were used and $\Delta_{mix} h = 1.6 \text{ kJ/mol}$ and $\Delta_{mix} s = 10 \text{ J/molK}$ were obtained from the fit.
A solution of Venlafaxine free base in toluene behaves almost as an ideal solution, when including the temperature dependence of the fusion enthalpy, which means that the solvent-solute interactions are very similar to the solvent-solvent and the solute-solute interactions. In case of heptane, a solvent molecule without a significant dipole moment, the intermolecular interactions are due to van der Waals forces. For Venlafaxine free base, a more polarizable molecule, the dipole moment is larger, but it also forms an intra-molecular hydrogen bond between the OH and the dimethylamino group. These two effects can account for the large $\Delta_{\text{mix}}h$ (26.5 kJ/mol) found from the solubility data fit. The solvent-solvent and solute-solute interactions are preferred compared to the interaction between solvent and solute molecules. In other words the mixing energy is positive and large.

For methanol, a solvent with a hydrogen bonded structure, the molecular properties are more similar to those of Venlafaxine free base; the solvent-solute interactions are more comparable to the solvent-solvent and the solute-solute interactions, resulting in a smaller $\Delta_{\text{mix}}h$ (1.6 kJ/mol) to fit the solubility data.

Remarkably, the solution of Venlafaxine free base in toluene, a molecule without a significant dipole moment and no possibility to form hydrogen bonds, behaves almost as an ideal solution; the observed and the calculated solubility fit very well and only a small $\Delta_{\text{mix}}h$ (0.5 kJ/mol) was necessary to fit the data, suggesting a strong $\pi-\pi$ interaction between the solvent and solute molecules.

By extrapolating solubility trendlines of the respective polymorphs in the ideal van ’t Hoff plots, transition temperatures can be calculated. This temperature for the transition of polymorph I to II is 35.7 ºC. For the transition of polymorph II to III this temperature is 51 ºC. When we apply equation (8) to calculate the transition temperatures for our system, using the thermodynamic data of table 3, we find a temperature for the transition of polymorph I to polymorph II of 28ºC, and 56ºC for the transition of polymorph II to form III. These calculated transition temperatures agree reasonably with the results of the solubility experiments.

### 3.6 Conclusions

The three forms of the free base of Venlafaxine were studied. All three forms were isolated and characterized using different techniques; DSC and X-ray powder diffraction and solubility experiments. From slurry experiments it was found that all the three forms have a distinct temperature range where they are stable, in other words there exists an enantiotropic relation.
between the three forms. The results of measuring DSC thermograms of a compound with different heating rates of 10 °C/minute and lower, show that several phenomena can be missed like solid-solid transitions and/or melt-recrystallizations. Although this is a well established fact, it is still common practice to measure DSC thermograms with heating rates of 10 °C/min or more.

The solubility curves of Venlafaxine free base in different solvents could be only described when the temperature dependence of $\Delta_{fus} h^*$ was included. This term, which is independent of the solvent, was determined by measuring the difference in heat capacity of the solid and undercooled melt of the solute. It was shown that using solubility data in the form of van ‘t Hoff curves to draw conclusions about the stability regions of two enantiotropic related polymorphs can lead to paradoxical situations if the temperature dependence of $\Delta_{fus} h^*$ is not considered. Although this temperature dependence is almost always neglected, the present results show that care should be taken in doing so, especially in the case of pharmaceutical compounds. These often have a large number of degrees of freedom due to the complex structure of the molecules, and consequently a non-negligible $\Delta c_p$ can be expected between the undercooled melt and the solid phase.

Finally, a recently derived expression for the transition temperature of two enantiotropic forms in terms of the heat of fusion and the melting temperatures of the forms, as determined by for example DSC measurements, was validated for Venlafaxine. The results agree reasonably with the experimental transition temperatures. This is remarkable because in case of the calculated transition temperatures the non-negligible temperature dependence of $\Delta_{fus} h^*$, as was found for Venlafaxine, was not taken into account.
3.1 References

7. Davey, R. J., Current Topics in Materials Science, (1982), 8, 429-479
14. US patent, 4,535,186
15. US patent, 6,197,828
16. WO patent 00/76955
17. WO patent 03/082806
Abstract

The unique behaviour of the active pharmaceutical ingredient Venlafaxine free base, used as an anti-depressant, with respect to polymorphism and chiral resolution is reported. Using several complementary techniques, three crystal structures of Venlafaxine were identified and isolated. All three structures are composed of virtually identical enantiomeric pure layers with different stacking modes. In the crystal structure with the highest melting point, the enantiomeric separation is complete, leading to a racemic conglomerate. The conglomerate can be grown from solution or via a solid-solid phase transition of the lowest melting racemic compound. Remarkably the crystal shape is conserved during the transition. The corresponding chiral resolution is achieved via a local melting process, allowing for a long range migration of the molecules between layers.
4.1 Introduction

Polymorphism, crystallization and chiral resolution are phenomena that are of great industrial relevance. Here we study a pharmaceutical compound, which combines these phenomena as a result of different stacking sequences of enantiopure layers of the compound in its various crystal structures.

The phenomenon of polymorphism, the ability of a substance to exist in two or more crystalline phases that differ in the arrangement and/or conformation of the molecules in the crystalline lattice, has important commercial and/or industrial implications in various fields\(^1\). The pharmaceutical industry and the photographic and imaging industry are two examples. Polymorphs consist of the same chemical compounds, but have different crystal packings, and as a result they can show different physical properties\(^2\). The occurrence of polymorphism is high, and McCrone stated that, in general, the number of polymorphic forms known for a given compound is proportional to the time and money spent in research on that compound\(^3\). Although the existence of different polymorphs of a certain compound is frequently observed, determining the conditions for their appearance usually is laborious. Most polymorphs are found as a result of serendipity rather than through systematic searches. The possibility to predict if and how many polymorphs exist of a compound is of great importance. Therefore, several computer programs with that aim have been developed, each of them with their specific advantages and limitations\(^4,5\). Most of these methods combine a random generation of crystal structures with an energy minimization step. An alternative way to predict the structures of possible polymorphs is the Derived Crystal Packing (DCP) approach developed by Coquerel et al\(^6\). The DCP procedure comprises two steps: first the extraction of periodic fragments (PF) from a known polymorphic form, secondly, three dimensional structures are generated by the application of symmetry operators. New phases are then built as low energy structures by minimizing the energy using molecular mechanics software. Recently the DCP method was successfully put into action to resolve the crystal structure of a metastable polymorph of Modafinil\(^7\).

Crystallization is often the final purification step for an active pharmaceutical ingredient (API). Until recently most of the API’s were marketed as racemates. As a result of regulatory aspects more and more effort is put into bringing medicines enantiopure to the market. In many cases this can be achieved by an enantioselective synthesis. Nevertheless, in an increasing number of cases this would involve too many synthetic steps for the process to be economically viable. Chiral separation by crystallization can be an alternative.
Crystalline racemates mainly belong to two classes. When both the enantiomers are present in equal amounts in the unit cell the compound is called a racemic compound or a true racemate, which is the most common type. In 5-10% of the cases racemic mixtures crystallize as racemic conglomerates, solids in which a single enantiomer is present in the unit cell. In these instances a spontaneous segregation takes place. Often one only considers a physical mixture of enantiopure crystals as a racemic conglomerate. However, systems in which crystals are built up from macroscopic enantiopure layers, racemic twins, can also be considered as racemic conglomerates. This phenomenon was described by Gervais et al.\textsuperscript{9}. They investigated a compound which crystallizes as a racemic conglomerate, but experienced a lot of trouble during separation by preferential crystallization. Isolated crystals, shaped as single crystals, showed no or almost no enantiomeric excess, a measure for the enantiomeric purity. This phenomenon was also observed by Green et al.\textsuperscript{10}. The initially considered (ordinary) macroscopic twinning along a growth direction was rejected as a result of dissolution experiments performed with the obtained crystals. Gervais et al.\textsuperscript{9} explained the phenomenon as the repeated formation of epitaxial layers (macro twinning) of crystals of the two enantiomers. Detailed study of the crystals revealed that the crystals were built up from thick alternate layers of R and S molecules, formed via an oscillating crystallization mechanism. An analogous case of spontaneous oscillating crystallization of R and S enantiomers was described by Potter et al.\textsuperscript{11} and Berfeld et al.\textsuperscript{12}.

All these cases are a special case of epitaxial crystal growth. Usually epitaxy is observed as the oriented nucleation and growth of crystalline layers of a compound on a specific crystalline surface of another compound. This will often lead to large stresses and strains, as a result of the lattice mismatch. However, in the case of the epitaxy of enantiomers, a layer of, for example, S molecules grows epitaxially on top of a crystalline substrate of R molecules, without any significant lattice mismatch as a result of the enantiomeric relation between the two crystal structures. Note that the two layers do not need to be mirror images of each other. Nevertheless, the similarity between the two enantiomerically related structures can lead to a very low interfacial stress, resulting in a relatively low barrier for the epitaxial nucleation and growth. Epitaxial nucleation of strongly related crystalline phases was recently also found for polymorphs, that is, different crystal structures of the same compound\textsuperscript{13,14}. In this case the metastable polymorph of a hormone was found to grow epitaxially on the stable polymorph. In later studies it was even found that the reverse process was also possible\textsuperscript{15}. Both polymorphic forms consisted of almost identical layered structures. For the metastable polymorph, with spacegroup P1, only one conformer of the molecule was present, while the stable polymorph, with spacegroup P2\textsubscript{1}, consisted of four layers, successive layers having a different conformer of
the molecule. Such a situation can be considered as a special case not easily covered by the DCP-approach, as it involves different conformers of the molecule. Furthermore, in this case the small structural difference between the layers resulted in a small barrier for epitaxial nucleation. The major difference with enantiomeric epitaxial crystallization, however, is the fact that the polymorphic epitaxial layers of the metastable polymorph were able to transform via a solution mediated mechanism to the stable polymorph structure. Giovannini et al\textsuperscript{16} describe a transition of a true racemate to a racemic conglomerate. A racemic dihydrate of Zopiclone is dehydrated forming a metastable anhydrate which, upon further heating, melts and the racemic conglomerate phase crystallizes out. A solid-solid transition of a racemic conglomerate to a true racemate is described by Mercier et al\textsuperscript{17}. Racemic conglomerate crystals of the compound \([\{\text{H}_3\text{N}(\text{CH}_2)_2\text{SS}(\text{CH}_2)_2\text{NH}_3\} \text{PbI}_5\} \cdot \text{H}_2\text{O}\) changed conformation of half of the disulfide moieties upon heating to 75 °C, leading to a true racemate. The transition back to the racemic conglomerate could also be accomplished. One has to note, however, that the chirality of "chiral disulfides" often results from configurationally chiral ligands linked to the chalcogenide atoms and that racemisation occurs in solution as a result of the relatively low barrier of rotation of the S-S bond between both enantiomeric conformers.

Here we study Venlafaxine free base, which shows both polymorphism as well as chiral resolution as a result of different stacking sequences of enantiopure layers of the compound. Venlafaxine is an antidepressant of the class of phenethylamines that inhibits the reuptake of serotonin, norepinephrine, and to a lesser extent, of dopamine. It is administrated orally as the hydrochloric acid salt and is marketed as a racemate. Both enantiomers are reported to be active. The common name for the compound Venlafaxine is: 1- [2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol. The molecular structure of the free base is given in figure 1.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{venlafaxine.png}
\caption{The molecular structure of Venlafaxine; * denotes the chiral carbon atom.}
\end{figure}

Two polymorphs of the free base were known, and a previously performed extensive study of the thermal behaviour of the free base yielded one new crystal structure\textsuperscript{18}. It turned out that an enantiotropic\textsuperscript{19} relation exists between all three crystal structures, implying that below a certain
transition temperature one form is stable, and above that temperature another one is stable. Form I is stable below 40 °C, form II between 40 and 50 °C and a third form above 50 °C. The present study of the structural relation between the three crystal structures shows that forms I and II are true racemates built up of sequences of enantiopure R and S bi-layers, and enantiopure double bi-layers respectively, and therefore true polymorphs while form III consists of alternate macroscopically thick layers of R and S molecules. Therefore, form III is an epitaxial racemic conglomerate and not a real polymorph in the strict sense of the definition. Furthermore, we investigate the metastable solid-solid transition (peritectoid) between the racemic compound form I and the racemic conglomerate, form III. Remarkably this transition is topotactical, as the shape of the crystal does not change during this racemate to racemic conglomerate transformation.

4.2 Experimental

Venlafaxine with a purity of 99.9%, according to High Pressure Liquid Chromatography (HPLC), was supplied by Synthon B.V.. The solvents (pa) were purchased from Aldrich and used without further purification. Crystals of the three polymorphs were obtained by cooling a solution of Venlafaxine in heptane saturated at reflux temperature. The various forms were obtained by quickly cooling the clear solution to a temperature for which the desired form is stable, and isolating the formed crystals at that temperature. When isolating single crystals of forms II and III, a layer of the low melting polymorph(s) is easily deposited on the crystal. To avoid the formation of these layers water of the right temperature was poured in the reaction flask to separate the hot heptane solution from the crystals as fast as possible. After filtration of the suspension, the water attached to the crystals was removed by drying the crystals in a vacuum desiccator using phosphorus pentoxide to trap the water.

It was difficult to obtain suitable single crystals of the high melting form III for two reasons. Firstly, crystallizing form III from solution often leads to very small crystals or an agglomerate of crystals. Secondly, form III is only stable above 50°C and because the solubility is very high at that temperature, 38 g of Venlafaxine “dissolves” in 10 g of heptane at 59°C. Moreover, the liquid layer surrounding the isolated crystals cools quickly during isolation, and the resulting high supersaturation easily leads to the formation of a layer of polymorph I and/or polymorph II on top of the crystals of form III. Seeding a supersaturated solution above 50°C with small crystals, isolated from a former experiment and washed to remove the layers of the other forms, resulted in the formation of large single crystals of form III.
Scanning Electron Microscope (SEM) photos were taken using a JEOL JSM 6330F Field Emission SEM. Optical rotations were measured with a Perkin Elmer 343 polarimeter at 589 nm and 20 °C. The polarimeter was used with the micro aperture set-up.

For the single crystal X-ray diffraction experiments measurements, single crystals were mounted in air on glass fibres. A course structure determination was performed at room temperature for each form. The final intensity data were collected at -65°C; only a small contraction of the unit cell was observed. A Nonius Kappa CCD single-crystal diffractometer was used (φ and ω scan mode) using graphite monochromated Mo-Kα radiation. Intensity data were corrected for Lorentz and polarization effects. For absorption correction the Siemens Area Detector ABSorption correction program (SADABS) was applied. All structures were solved by the program CRUNCH and were refined with standard methods using SHELXL97 with anisotropic parameters for the nonhydrogen atoms. The hydrogens attached to the methyl and hydroxy groups were initially refined as rigid rotors to match maximum electron density in a difference Fourier map and were freely refined subsequently. All other hydrogens were initially placed at calculated positions and were also freely refined subsequently. For crystals of the enantiopure compound as well as crystals of form III (grown from solution or obtained by conversion of crystals of polymorph I after heating) the absolute structures could not be determined reliably by refinement of the Flack parameter in SHELXL97. However, based on the assignment of the chirality of Venlafaxine as in relation to the optical rotation as described by Yardley et al., we assigned the R conformation to the structure of the enantiopure compound.

Cross Polarization Magic Angle Spinning (CPMAS) 13C solid state NMR spectra were acquired on a 400 MHz Chemagnetics Infinity spectrometer. A 4 mm and a 3.2 mm double resonant probe were used, tuned to 100.58 MHz for carbon and 399.95 MHz for protons. Measurements on polymorphs I, II and the as-grown form III were done on the 4 mm probe with 8.0 kHz MAS. Here, variable amplitude cross polarisation (VACP) with a contact time of 2 ms was used with a radio frequency (RF) field strength of 64 kHz on protons and 56 kHz on carbon and a +1 to -1 kHz linear ramp on protons. During acquisition the protons were decoupled with a two pulse phase modulation (TPPM) pulse sequence with a 110 kHz RF field, a pulse duration of 5.3 μs and a phase modulation of 15 degrees. Partially dissolved form III and enantiopure form III were measured with the 3.2 mm probe at a spinning speed of 12.5 kHz. VACP was used with a 62 kHz field with a +/- 0.6 kHz ramp on 1H, and a 62 kHz field on 13C. For proton decoupling the continuous modulation (CM) scheme was used with a modulation amplitude of 0.12 radians, a period of 8.4 μs and an RF field strength of 110 kHz. Peak intensities were
obtained by deconvolution of the spectra using a Voigt lineshape. The data were processed using the MatNMR processing package which runs under Matlab\textsuperscript{29}.

Molecular energies, lattice energies and surface energies were calculated using the Cerius\textsuperscript{2} modelling environment\textsuperscript{30}. The crystal structures were minimized using the Dreiding force field with 3D-Ewald summation for the Coulomb contribution. Charges were determined using a Restricted Electrostatic Potential (RESP) charge fitting scheme\textsuperscript{31}. The Van der Waals interactions were calculated using a spline function with on and off-distances at 12 and 13 Å respectively. To calculate the lattice energy, the energy of individual molecules with the bulk conformation was calculated and subtracted from the energy found for the crystal structures. For the calculations of surface energies the crystal structure was cleaved. Using the same settings as for the bulk crystals, only now with a 2D-Ewald summation, the surface energies were calculated by subtracting the lattice energy of the full crystal from that of the cleaved semi-infinite crystals without relaxation.

4.3 Results and Discussion

4.3.1 X-ray single crystal structure determination

The crystal structures were determined using single crystal X-ray diffraction. Polymorphs I and II crystallize in the monoclinic spacegroups P2\textsubscript{1}/n and P2\textsubscript{1}/c, respectively. Note, that the latter spacegroup represents an alternative setting of the former one. Form III crystallizes in an orthorhombic structure, spacegroup P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}. For an overview of the crystal data of the three polymorphs see table 1.

The crystal structure determinations of forms I, II and III showed that all structures are built up from similar enantiopure layers parallel to the (001) surface. The crystal structure of polymorph I is in agreement with the previously published structure\textsuperscript{32}(Cambridge Crystal database reference code; OCALAG). In case of polymorph I, alternating layers of R and S molecules make up the structure, for polymorph II alternating bi-layers of R and S molecules are present and for form III only layers of one enantiomer make up the complete crystal packing. Polymorphs I and II are true racemates because R and S molecules are stoichiometrically, ratio (1:1), present in the unit cell. For the unit cells of forms I, II and III see figure 2.
Figure 2: Projections of the unit cells of forms I, II and III of Venlafaxine; for form I and III the a and c axes and for form II the b and c axes are indicated.
Table 1: Crystal data of forms I, II and III (grown from solution), III (after transformation) and of one of the pure enantiomers of Venlafaxine free base, all structures were measured at -65 °C.

<table>
<thead>
<tr>
<th></th>
<th>space group</th>
<th>a (Å)</th>
<th>b (Å)</th>
<th>c (Å)</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Z</th>
<th>V (Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form I</td>
<td>P2₁/n</td>
<td>8.82</td>
<td>21.61</td>
<td>90</td>
<td>92.22</td>
<td>90</td>
<td>90</td>
<td>4</td>
<td>1586.4</td>
</tr>
<tr>
<td>Form II</td>
<td>P2₁/c</td>
<td>8.84</td>
<td>8.27</td>
<td>43.75</td>
<td>90</td>
<td>90.97</td>
<td>90</td>
<td>8</td>
<td>3198.6</td>
</tr>
<tr>
<td>Form III (grown)</td>
<td>P2₁2₁2₁</td>
<td>8.22</td>
<td>8.86</td>
<td>22.27</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>4</td>
<td>1622.7</td>
</tr>
<tr>
<td>Form III (transformed)</td>
<td>P2₁2₁2₁</td>
<td>8.23</td>
<td>8.87</td>
<td>22.33</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>4</td>
<td>1630.5</td>
</tr>
<tr>
<td>Pure enantiomer</td>
<td>P2₁2₁2₁</td>
<td>8.15</td>
<td>8.80</td>
<td>22.30</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>4</td>
<td>1599.3</td>
</tr>
</tbody>
</table>

The crystallographic relationship between molecules in the enantiopure layers of forms I, II and III differ slightly. In form I the molecules in the enantiopure (mono)layers are related by translational symmetry in the a- or b-direction or by a twofold screw axis in the b-direction. In form II the molecules in the enantiopure layers are related by translational symmetry in the a- and b-direction or by a pseudo-twofold screw axis in the a-direction. In form III the molecules in the layers are related in the same way as in form I. The consequence is that the molecular arrangement in the a-direction of form I is similar to the arrangement in the b-direction of form II and similar to the a-direction of form III. And, vice versa, the molecular arrangement in the b-direction of form I is similar to the arrangement in the a-direction of form II and similar to the b-direction of form III. This relationship is also visible from the unit-cell parameters of the three forms. The crystallographic relationship between layers consisting of different enantiomers is in both form I and II the inversion symmetry. The relation between layers of the same enantiomer that make up bi-layers in the structures of forms II and III is a two-fold screw axis, for form II in the b-direction and for form III in the a-direction. This means that inverting an enantiopure layer must yield a resulting layer that is structurally very similar to a layer obtained by applying a two-fold screw axis (in the proper direction), at least with respect to the structure of the interface between to enantiopure layers. This structural resemblance is visible in figure 3 in which an overlay is shown of R and S layers that have a similar contact to other enantiopure layers. Clearly the overall structure of the interfaces is very similar although the layers are made up of different enantiomers. The crystal structure of form III pointed to the presence of only one enantiomer, although we used the same racemic starting material as for the crystallizations of
polymorphs I and II. This indicates the formation of a racemic conglomerate, as will be discussed below.

Figure 3: Overlay of the unit cells of polymorph I (green), polymorph II (yellow) and form III (red).

4.3.2 Solid State NMR characterisation

Using $^{13}$C solid state NMR all three forms were investigated. Figure 4 shows the spectrum of a microcrystalline powder of a pure enantiomer grown from a heptane solution. These crystals have the same structure as form III. The assignment of the carbon spectrum was done based on chemical shifts and dipolar dephasing experiments (not shown here)$^{33}$. Peaks stemming from carbon 6 and 7a/b are somewhat broader due to the residual dipolar coupling to $^{14}$N. Stacking effects are clearly present in the spectrum as carbon atoms that would be equivalent in solution now have different chemical shifts (up to 10 ppm for carbon 10a/b).
Polymorphism and migratory chiral resolution of the free base of Venlafaxine

Figure 4: $^{13}$C CPMAS NMR spectrum of Venlafaxine in form III, grown from an enantiopure solution. The numbers on top of the peaks correspond to the carbon atom labels in the molecular structure.

Figure 5 displays the $^{13}$C spectra of the microcrystalline powders which were grown from a racemic solution. Polymorphs I and II are shown in 5a and 5b while the spectrum of the as-grown form III and the partially dissolved form III are displayed in 5c and in 5d. Clear differences can be seen between the different forms. One can observe three sets of chemical shift; those shared between polymorphs I and II, those shared by forms II and III and those shared by all 3 forms. Combining this with the crystal structures, it is straightforward to conclude that the chemical shifts shared between polymorph I and II are associated with the stacking of 2 different enantiomer layers, i.e the RS interface between an R bi-layer and an S bi-layer, while those shared between II and III are unique for the stacking of like enantiomer layers, i.e. an R bi-layer stacked on another R bi-layer (or an S bi-layer on another S bi-layer). The difference in chemical shift between an unlike interface (RS) and the like interface (either RR or SS) are most noticeable for peaks stemming from carbon 1 and 12 and a little less for the other 6-ring carbons (2a/b, 3a/b and 4). From the crystal structure, see figure 2, these are actually the carbon atoms that are lying at the interface of the bi-layers. Quantification of $^{13}$C CPMAS spectra has to be done with care and is usually not straightforward. In this case, however, no differences in CP dynamics between the different forms could be observed. For polymorph II we know that half
the peaks originate from the like interface and half from the unlike interface. Inspection of the spectrum of form II shows that intensities of for instance the two peaks of carbon 12 appear in a 1:1 ratio. Hence, quantification of the relative amounts of the layer types based on the VACP measurements was possible.

Figure 5: $^{13}$C CPMAS NMR spectra of the different forms of Venlafaxine, grown from a racemic solution: a) form I, b) form II, c) form III, as-grown, and d) form III, rinsed. Chemical shifts are listed in table 2. Spinning sidebands are indicated with an asterisk. MAS speeds were 8 kHz in a, b and c and 12.5 kHz in d.
Table 2: $^{13}$C Chemical shifts for the different forms, carbon atoms are numbered according to figure 4.

<table>
<thead>
<tr>
<th>Carbon Atom</th>
<th>Form I $\delta$ (ppm)</th>
<th>Form II $\delta$ (ppm)</th>
<th>Form III $\delta$ (ppm)</th>
</tr>
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<tr>
<td>2 a/b</td>
<td>22.7</td>
<td>22.7</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>23.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28.2</td>
<td>28.3</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.0</td>
<td></td>
</tr>
<tr>
<td>3a/b</td>
<td>31.5</td>
<td>31.5</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>37.6</td>
<td>37.5</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.0</td>
<td></td>
</tr>
<tr>
<td>7a/b</td>
<td>43.6</td>
<td>43.6</td>
<td>43.4</td>
</tr>
<tr>
<td></td>
<td>47.8</td>
<td>48.0</td>
<td>48.3</td>
</tr>
<tr>
<td>5</td>
<td>52.3</td>
<td>52.4</td>
<td>52.5</td>
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</tr>
<tr>
<td></td>
<td>75.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10a/b</td>
<td>109.1</td>
<td>109.1</td>
<td>109.2</td>
</tr>
<tr>
<td></td>
<td>119.1</td>
<td>119.3</td>
<td>119.2</td>
</tr>
<tr>
<td>9a/b</td>
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<td>130.1</td>
<td>129.9</td>
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<tr>
<td>11</td>
<td>159.6</td>
<td>159.9</td>
<td>156.0</td>
</tr>
</tbody>
</table>

4.3.3 Dissolution experiments of grown crystals

The X-ray data of form III of Venlafaxine pointed towards the formation of a racemic conglomerate, with single crystals composed of enantiopure fragments. Furthermore, both the X-ray crystallography and the NMR results indicate that the structure of form III grown from the racemic solution is identical to the one grown from an enantiopure solution.

A possible explanation of the observation is that somehow during the process an enantiomeric resolution took place, and separate crystals of R and S were formed. To examine this possibility several large single crystals were isolated, and the optical rotation of the single crystals was measured separately. In all cases the optical rotation was negligible. From this result it was concluded that (almost) equal amounts of both enantiomers were present in the crystals.

Therefore, during low supersaturation conditions the single crystals have to grow via the successive formation of enantiopure fragments. A mechanism, which accounts for the formation of single crystals consisting of macroscopic layers of enantiopure fragments, via oscillating crystallization, was already described in the literature$^{9,11}$. During the growth of a face only one
enantiomer is inserted and as a result of that the local supersaturation of the other enantiomer is increasing until a certain threshold value. Then nucleation of the other enantiomer on top of the already existing enantiopure fragment results in the growth of a new enantiopure fragment of the opposite handedness. Gervais et al. were able to overcome the problem of the formation of crystals by slowly stirring the solution. In that case the diffusion rate is large enough to keep the supersaturation of the other enantiomer below the threshold of 2D nucleation. Instead of trying to avoid this oscillating growth behaviour, we study the composition of the as-grown crystals in more detail.

To further investigate the composition of the single crystals selective dissolution experiments were performed as proposed by Toyokura et al.\textsuperscript{34} which offers a simple method to study the enantiomeric composition of the single crystals of form III. A crystal of form III which was grown from a heptane solution containing racemic Venlafaxine, was added to a saturated solution of one of the pure enantiomers in heptane at room temperature. Assuming ideal solubility mainly fragments containing the R enantiomer should dissolve in a solution saturated with the S enantiomer at a temperature for which form III crystals are metastable. The dissolution process was followed in situ using optical microscopy. After several hours the crystals were isolated and analysed using scanning electron microscopy (SEM). In Figure 6 the left image gives an overview of a crystal before etching. A striped pattern is visible on top of the crystal, suggesting a fragmented composition of the crystal present before etching. This pattern is shown enlarged in the insert and can be compared with the SEM image of a crystal after etching, shown on the right in Figure 6.

![Figure 6: SEM photographs of single crystals of form III before (left; scale bar = 1mm) and after (right; scale bar =0.1 mm) a dissolution experiment in a heptane solution saturated with one of the enantiomers. The insert shows a part of the crystal surface with the same magnification as for the image on the right. The orientation of the b and c-axis are indicated.](image-url)
Some crystals were left in the solution saturated with the S enantiomer for 16 hours. The optical rotation of the isolated fragments showed an enantiomeric excess of more than 80%. This result confirms that mainly fragments containing R enantiomers dissolve. From a comparison of the SEM images it is obvious that intermediate layers of the crystal, with opposite handedness, dissolved during the dissolution experiment. Moreover the images confirm that that the as-grown single crystals of form III are composed of enantiopure fragments with a thickness of 10-50 \( \mu m \). These fragments contain one of the enantiomers in a large excess.

From the NMR data one can also determine the average layer thickness through the relative ratio of the peaks unique for the unlike (RS) interface and the peaks unique for the like interface (RR and SS). The as-grown form III (figure 5c) shows some low intensity peaks that are characteristic for the RS interface. Based on the peak intensities one arrives at an average like layer thickness between 8 and 10 interfaces (comparable to 4-5 stacked unit cells of form III). This seems rather thin, compared with the layer thickness as seen in the SEM images. However, as was mentioned before, the isolation of single crystals of form III caused some difficulties, the result of the high solubility of Venlafaxine in heptane. Crystals isolated from the solution are covered with a layer of liquid with a high concentration of Venlafaxine. A layer of polymorph I and or II can crystallise on the surface of the crystal of form III before this liquid layer can be removed. Form I and II both contain RS interfaces and this would explain the existence of the low intensity peaks in figure 5c. To examine this idea, single crystals of form III were added to a flask with heptane and left there for some time to dissolve the outer layer, the part of the crystal were RS interfaces are likely to be present. After isolation and drying of the crystals the spectrum in figure 5d was recorded. The intensity of the RS interfacial peaks are much reduced, leading to an average enantiopure layer thickness of 40 layers (equivalent to 20 units cells of form III stacked along c). This is still much thinner than the 10-50 \( \mu m \) (which would be 5000 to 25000 unit cells) which the SEM images suggested. It should be noted, however, that the layer thickness is an average over the entire crystal, enantiopure layers could be separated by thin layers of form I and/or II or single layers of one enantiomer can be interspersed within a thick layer of the other enantiomer. These thin layers of what would essentially be a single unit cell of form I would not show up in the X-ray diffraction, nor in the etching experiments.

4.3.4 Solid-solid transition of polymorph I to III

In a related paper\(^\text{18}\) the solid-solid transition of form I to III was followed in time using DSC, XRPD and solid state NMR. Especially the XRPD and NMR measurements showed a gradual transformation between the polymorphs. To realize such a transformation in the solid
state complete layers of R and/or S molecules have to be transferred, a process that on first sight seems very unlikely, see Figure 7.

*Figure 7: Annealing of a crystal of polymorph I at 75°C for 6 days leads to its transformation to form III. The R and S enantiomers need to migrate over surprisingly large distances during this process.*

For NMR, XRPD and DSC finely powdered material was used. Taking into account the large surface area and the acquired stress resulting from grinding, the solid-solid transition is imaginable. The question then arises, whether this transition could also be established in a single crystal. A comparable experiment was performed using a single crystal of polymorph I (dimensions approximately 2 mm in all directions). With single crystal X-ray diffraction the crystal structure was confirmed. The single crystal was subsequently transferred to a calorimeter and slowly heated to 74 °C which is 4 degrees below the melting temperature of form III, that is the eutectic temperature of the racemic conglomerate, and kept at that temperature for 6 days and then cooled to room temperature. According to single crystal structure determination, the starting material, polymorph I, was converted to form III; remarkably the shape of the crystal had not changed.

To study the layered structure of this crystal in more detail an annealed crystal of polymorph I was etched in a similar way as for crystals of form III by adding it to a saturated solution of one of the pure enantiomers. SEM photos were taken from the isolated crystals after the etch experiment, see Figure 8.
Although the solution might lead to a reconstruction of the layered structures, it is clearly visible from the figure that the layers are built up in a mosaic fashion, contrary to the layered fragments of the etched crystals of form III as grown from solution, see Figure 6.

The only way to transform form I into III is by the migration of molecules, inversion of the chiral centre is impossible. Furthermore, diffusion of complete layers in the solid state is also not very likely. To account for this phenomenon it is suggested that diffusion has to occur via a kind of molten phase. A possible explanation is local melting as a result of crystal defects, impurities and/or stress, several degrees below the melting point of the bulk solid. During the annealing a thin layer melts and after nucleation an R or S domain starts to grow. Like in the experiment of Gervais et al.⁹, after some time the supersaturation of the other enantiomer has reached the threshold for 2D nucleation and after nucleation an enantiopure domain starts to grow epitaxially on top of the underlying layer. For the present case it is probable that the pre-melting process starts at different spots in the crystal simultaneously. At some of these places R starts to nucleate and at others S will start to nucleate. In this way no enantiopure layer, as for the crystals of form III grown from solution, but a mosaically composed layer is formed for the annealed crystal. The molten fronts move through the crystal resulting in a crystal composed of enantiopure domains. The thickness of the domains is of the same order as for the layers of the grown crystals of form III.

The difference between the volumes of the unit cells of the grown (1623 Å³) and the transformed form III (1630 Å³) confirm the view resulting from the above described experiment. During the transformation of polymorph I to form III the packing efficiency of the formed
mosaic crystal fragments differs slightly from that of grown crystals of form III, resulting in a slightly larger unit cell. This is also validated by the temperature factors of determined crystal structures. For the transformed form III they are slightly larger.

4.3.5 Molecular modelling

Looking at the remarkable solid-solid transition one can ask what is the driving force for this racemate-to-conglomerate transition. As the structures of the individual layers for all three forms are virtually the same the interactions between molecules inside a layer can be considered equal. For the inter-layer interactions it can be concluded that at lower temperatures, for which form I is stable, R-S interfaces are favored, whereas at higher temperatures (form III) R-R (and S-S) interfaces are dominant. Form II can be considered as an intermediate phase as 50 % of its interfaces are R-S and the remaining 50 % are R-R and S-S.

To get an estimate of the energies involved in the interfacial energies were determined using molecular mechanics calculations of the lattice energies of a bulk crystal and a cleaved one. The results of these calculations are summarized in table 3. The lattice energies are the same within 0.4 kcal.mol\(^{-1}\) apart, thus within the error margin (roughly 2 kcal/mol) of the calculations. The differences in surface energy between form I and II is for the R-S layers 0.8 kcal.mol\(^{-1}\), which may indicate a small enthalpic favour for form I, which is in accordance with the fact that form I is formed at low temperatures. The difference in R-R surface energy for form II and III, however, is 0.7 kcal.mol\(^{-1}\), which hints at a small enthalpic favour for form III forming over form II, at lower temperatures, which is in contrast with the observations.

Entropic contributions usually dominate the polymorphic stability more than the enthalpic contributions.\(^{10}\) Assuming that the transition between the enantiotropically related forms are mainly entropy driven, in other words assuming that the enthalpies and entropies of the polymorphs differ but are only weakly dependent on temperature, the order of enthalpies is \(H_I < H_{II} < H_{III}\), and the order of entropies follows \(S_I < S_{II} < S_{III}\). The entropy has two contributions, a conformational part and a vibrational part. The conformational part is the same for all the three forms, while the vibrational part of the entropy will differ. Limiting our attention again to the interlayer interactions and form III having only interactions between R and R (or S and S) layers, this leads to the conclusion that there is more vibrational freedom in these interactions as compared to interactions between R and S layers.
Polymorphism and migratory chiral resolution of the free base of Venlafaxine

Table 3: Calculated lattice and interface energies for forms I, II and III.

<table>
<thead>
<tr>
<th></th>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal [kcal.unitcell⁻¹]</td>
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<td>161.49</td>
<td>61.93</td>
</tr>
<tr>
<td>Molecule 1 [kcal.mol⁻¹]</td>
<td>44.4</td>
<td>48.44</td>
<td>43.27</td>
</tr>
<tr>
<td>Molecule 2</td>
<td>-</td>
<td>47.64</td>
<td>-</td>
</tr>
<tr>
<td>Lattice energy [kcal.mol⁻¹]</td>
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<td>-27.85</td>
<td>-27.78</td>
</tr>
<tr>
<td>R-R surface</td>
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</tr>
<tr>
<td>R-R excess energy</td>
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<tr>
<td>R-S excess energy</td>
<td>15.03</td>
<td>15.85</td>
<td>-</td>
</tr>
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</table>

4.4 Conclusion

Three different forms of the antidepressant Venlafaxine were identified and their crystal structures determined. The crystals of the three forms are composed of enantiopure layers. Form I consists of alternating bi-layers of R and S enantiomers while form II consists of two R bi-layers alternating with two S bi-layers. Single crystal X-ray diffraction experiments combined with dissolution experiments showed that crystals of form III are racemic conglomerates, composed of a stacking of relatively thick layers of R and S enantiomers. Therefore in Venlafaxine a unique combination of phenomena, polymorphism and chiral separation, is encountered in a single compound. The overall structure of the interfaces between the bi-layers in the three polymorphs is very similar, the sequence of the bi-layers, however, differs considerably for the different forms. From ¹³C solid state NMR experiments it emerged that each type of layer interface (RS and RR/SS) has a unique spectral signature. From these experiments the average (enantiopure) layer thickness for form III was calculated. Although this led to much thinner layers compared with the layer thickness found from SEM images, showing that etching of the crystals in the presence of one of the enantiomers resulted in lamellae of 10-50 μm thickness, the results could be compared taking into account that the etching experiment will not reveal the thinnest lamellae present. Molecular modelling studies show that the differences in lattice enthalpy between the three structures is of the order of 1 kcal/mol, implying that the transitions between the three forms are entropy driven. Furthermore it was shown that a single crystal of Venlafaxine form I readily undergoes a topotactical solid-solid phase transition.
to form III as the habit of the crystal did not change during this transformation. The experiments indicate that local melting is the underlying mechanism for this remarkable transformation as long range migration of molecules in the solid state needed to accomplish this transformation seems highly unlikely. Further investigations to gain insight in the dynamics of this process are underway.

**Acknowledgments:**

We would like to thank B. van Gestel, J.W.G. Janssen, A.A.K. Klaassen, and J.W.M. van Os for NMR assistance, and G.-J. Janssen for SEM measurements.
4.5 References

29 MatNMR is a toolbox for processing NMR/EPR data under Matlab and can be freely downloaded at http://matnmr.sourceforge.net
30 Cerius2 User Guide; Accelrys Inc.: 9685 Scraanton Road, San Diego, CA, USA, (1997).
32 Cambridge Structural Database (OCALAG).
Chapter 5

EXPERIMENTAL AND COMPUTATIONAL MORPHOLOGY OF THREE POLYMORPHS OF THE FREE BASE OF VENLAFAXINE.
A COMPARISON OF MORPHOLOGY PREDICTION METHODS.

Abstract

In this paper the experimental and the computational studies of the morphology of three polymorphs of the free base of Venlafaxine ((N,N-dimethyl)-2-(1-hydroxy cyclohex-1-yl)-2- (4-methoxyphenyl)-ethylamine) are reported. The morphology of all polymorphs has been predicted using the Bravais-Friedel-Donnay-Harker method, the attachment energy method and kinetic Monte Carlo growth simulations and these predictions have been compared with experimental observations. The Monte Carlo simulations allow for a detailed simulation of the growth process, including driving force and growth mechanism, which leads to a semiquantitative prediction of the growth morphologies of all three phases.

For phase I two distinct growth habits are found experimentally under the same conditions. This is explained by the occurrence of a spiral growth mechanism in one of the two, which was observed using AFM and which is also supported by the Monte Carlo simulations. The habit of phase II could only be explained from simulations when a spiral growth mechanism is assumed; the shape of phase III could not be modeled accurately from the Monte Carlo simulations. Although the shape of the crystal is reproduced accurately, some of the indices of the faces predicted are not in agreement with the indices measured. The deviations are interpreted to be due to the presence of domains in the crystals as a result of the layered structure.
5.1 Introduction

The study of polymorphism of pharmaceutical compounds is an important part in the development of a new drug\(^1\). As each polymorph has different metabolical characteristics, often only one polymorph can be used in a drug formulation\(^2\). Knowledge of the polymorphs and their relative stabilities is therefore indispensable for the development of drugs that are administered in crystalline form. Apart from formulation issues, each new polymorph can be patented, so also for protection of intellectual property adequate knowledge of polymorphism is needed.

Polymorphs can have different crystal morphologies, because morphology is largely determined by the crystal packing at the molecular level and the crystallization process. It is desirable to be able to exert control over the morphology, because it determines among other things the bioavailability and the processability of the compound. The bioavailability depends on the dissolution rate of the crystals, which is in turn partly determined by the crystal morphology. Concerning processability, it can generally be said that block-like crystals habits are desired over plate-like crystals and needles because the latter forms tend to block filters, are more difficult to dry and give problems during tabletting.

Crystal morphology can be predicted computationally using various methods of different complexity and demand for computing power. Well-known approaches are the method developed by Bravais, Friedel, Donnay and Harker (BFDH) based on the interplanar distances of crystallographic orientations\(^3,4\) and the method developed by Hartman and Perdok\(^5,6,7\) and later by Hartman and Bennema\(^8\), which uses the concept of the crystal graph to determine the slice and attachment energies of crystallographic orientations, and relates these energies to the growth rate of the orientations.

The development of detailed crystal growth simulations, for instance using Monte Carlo techniques, has been facilitated by the ongoing development and availability of ever faster computers. A recent development is a program called Monty with which growth simulations can be performed for arbitrary crystal structures in any crystallographic orientation as a function of the driving force for crystallization\(^9\).

Here the results are presented of a morphology prediction study for the three polymorphs of Venlafaxine using all three methods. A comparison with the morphologies observed experimentally is made. It is found that the predictions are semi-quantitative for phases I and II; for phase III the overall shape is reproduced, but some predicted indices do not match the indices observed experimentally.
5.2 Venlafaxine

Venlafaxine is a bicyclic phenylethylamine-based anti-depressive drug, which is believed to work by simultaneously blocking the re-uptake of neuronal norepinephrine and serotonin\textsuperscript{10}. The Venlafaxine molecular structure is shown in Figure 1. It is administered as a racemic mixture, and the crystals of this mixture are the subject of this study.

![Molecular structure of Venlafaxine.](image)

Recently it has been found that the free base of Venlafaxine has superior processing characteristics over the hydrochloric acid salt, which is marketed worldwide. The free base is easier to handle as it is less aggressive towards equipment etc. compared to the hydrochloric acid salt. Also, the free base shows a low solubility and low dissolution rate in water, and is therefore suitable for the formulation of extended release dosage forms\textsuperscript{11}. In the study of the free base, three polymorphs have been found, which are enantiotropically related\textsuperscript{12}. Phase I is stable at room temperature, and can be converted into phase II above approximately 40ºC. Phase I and phase II can be converted into phase III above 60ºC.

It can be seen from Figure 2 that the crystal structures\textsuperscript{12} of the three polymorphs are strongly related. When viewed along the \(b\)-axis, the unit cell of phase I consists of four molecules whose methoxyphenyl rings are oriented in a parallel fashion. In the crystal structure of phase III, the molecules stack with the methoxyphenyl rings in mutually perpendicular orientation. In the crystal structure of phase II these patterns of phase I and III are both incorporated; phase II can be thought of to be built of \{002\} layers of phase I and phase III. It must be noted, however, that this comparison only holds at a conceptual level, as due to packing details the crystal structures can not be overlaid exactly.
Figure 2: Crystal structures of phase I (left), phase II (middle) and phase III (right). The packing similarities can be seen, conceptually, as phase II being built up of layers of phase I and III.

Thus all polymorphs are built up of two types of layers, and each layer consists of enantiopure molecules (R or S). The polymorphs differ in the way that the R- and S-layers are stacked. Phases I and II are racemates, having spacegroup $P2_1/n$ and $P2_1/c$ respectively. Phase I stacks with alternating R- and S-layers; Phase II with two R-layers followed by two S-layers.

Interestingly, phase III has spacegroup $P2_12_12_1$, a chiral spacegroup. Phase III can, however, be obtained from racemic solutions. It was shown using enantiomeric etch experiments\textsuperscript{12}, that phase III is a conglomerate, built up of enantiopure macroscopic R- and S-layers: upon immersion of a crystal of phase III in a supersaturated solution of one enantiomer, the other enantiomer is etched away, and $\mu m$-thick layers of enantiopure material are left. The enantiomeric enrichment of the remaining solid was proven by determination of the optical rotation of a crystal dissolved after etching.

As all polymorphs are enantiotropically related, the solid state conversion of phase I and II to phase III at higher temperatures is very remarkable, as that implies large movements of
molecules from a molecularly racemic configuration to an enantiomeric conglomerate. Still, the etch experiments showed that a crystal that had been transformed from phase I to phase III is indeed a conglomerate of enantiopure material. For further details, the reader is referred to the work of van Eupen et al$^{12}$.

### 5.3 Morphology prediction

Generally speaking, growth morphologies can be constructed using a kinetic Wulff construction. In this construction the distance $r_{hkl}$ of a face $(hkl)$ from the origin of the crystal is taken to be linearly related to the growth rate $R_{hkl}$ of that face.

$$r_{hkl} \propto R_{hkl}$$

(1)

By constructing a three-dimensional set of faces perpendicular to the crystallographic orientations $(hkl)$ the growth morphology can be constructed if the growth rates of the faces are known. Different theories have been developed to derive or compute expressions for the growth rate $R_{hkl}$. Three of these will be discussed below.

#### 5.3.1 BFDH method

Although being an approach for predicting equilibrium morphology, the BFDH method is commonly used to relate the growth rate of a crystallographic orientation to the inverse of the interplanar distance $d_{hkl}$. The relevant indices $(hkl)$ are those allowed by the spacegroup selection rules. The distance from the center of the crystal to a surface $(hkl)$ is then given by

$$|r_{hkl}| = \frac{1}{d_{hkl}}$$

(2)

In retrospect, the rationale behind the method is that the larger $d_{hkl}$ is, the larger the energy content of the growth layer will be, and therefore the smaller the growth rate of the corresponding orientation will be. This limits the predictive power of the BFDH method in the case of anisotropic crystal structures. The determination of the values for $d_{hkl}$, however, is trivial once the space group and lattice parameters are known, making the BFDH a very fast method.
5.3.2 Hartman Perdok theory and the determination of the crystal graph

In the extension of the Hartman-Perdok theory by Hartman and Bennema, the concept of the crystal graph is introduced\(^8\). The crystal graph is a mathematical representation of the crystal structure as an infinite three-dimensional graph, in which the graph vertices represent the growth units and the graph edges represent the interactions between the growth units.

The crystal graph is used to determine Periodic Bond Chains (PBCs). PBCs are uninterrupted chains of bonds between symmetry-related growth units with an overall periodicity \([uvw] = ua + vb + wc\), \((u, v, w \in \mathbb{Z})\) of the primitive lattice, which contains no other lattice translation. PBCs are stoichiometric with respect to the contents of the crystal unit cell. Combinations of two or more intersecting PBCs in non-parallel directions in a growth layer define so-called connected nets.

Crystallographic orientations that have connected nets are likely to determine the growth morphology of the crystal\(^1\(^3\). In the Hartman-Perdok theory, the attachment energies of the connected nets in all crystallographic orientations determine the growth morphology. The bonds that are present in a crystal slice together make up the crystal's slice energy; the other bonds define the slice attachment energy. Together, they are equal to the crystallization energy:

\[
\begin{align*}
E_{\text{att}}^{\text{hkl}} &= \sum_i \phi_{\text{hkl}}^{i,\text{att}} \\
E_{\text{slice}}^{\text{hkl}} &= \sum_i \phi_{\text{hkl}}^{i,\text{slice}} \\
E_{\text{cryst}} &= E_{\text{att}}^{\text{hkl}} + E_{\text{slice}}^{\text{hkl}}
\end{align*}
\]  

Once the attachment energies for all orientations that have one or more connected nets have been computed, the attachment energies are related to growth rates \(R_{hkl}\) as

\[
R_{hkl} \propto E_{hkl}^{\text{att}} = E_{\text{cryst}} - E_{hkl}^{\text{slice}}
\]  

allowing for the prediction of the attachment energy morphology. The attachment energy method can thus be considered as a refinement of the BFDH method using the slice energy as the parameter determining the growth. The BFDH method uses the interplanar distance, which is merely a geometrical parameter.
5.3.3 Monte Carlo growth simulations

The methods described above give a reasonable approximation of the growth morphology in several cases. It is well-known, however, that the growth morphology depends on the driving force (i.e. the solution supersaturation) and growth mechanism during growth of the crystal\textsuperscript{14}. This is not taken into account when a morphology is calculated using the BFDH approach nor when based on the Hartman-Perdok theory. Recently the program Monty was developed by Boerrigter et al. that is able to perform growth simulations on any crystal structure in any crystallographic orientation\textsuperscript{9}.

In these simulations a box with lateral periodic boundary conditions is prepared in a certain crystallographic orientation ($hkl$) on which particles may attach and detach with the following probabilities:

\[ P_{ij}^+ = \exp \left( -\beta \lambda \left( U_{ij} - \Delta U_{ij} \right) - \beta \left( \lambda - 1 \right) \Delta \mu \right) \]
\[ P_{ij}^- = \exp \left( -\beta \left( \lambda - 1 \right) \left( U_{ij} - \Delta U_{ij} \right) - \beta \lambda \Delta \mu \right) \]

where $ij$ denotes a particle $i$ having a bonding configuration $j$ with surface bonding energy $U_{ij}$ and $\beta = (k_B T)^{-1}$. These probabilities ensure microscopic reversibility at all times. The probabilities allow for a choice of the parameter $0 \leq \lambda \leq 1$, which controls the contribution of $\Delta \mu$ vs. the surface bonding $\Delta U_{ij}$ to the probabilities. When $\lambda = 0$, the attachment probability, $P_{ij}^+$ is completely determined by the driving force $\Delta \mu$ and the detachment probability $P_{ij}^-$ is completely determined by the surface bonding energy $\Delta U_{ij}$. This probability scheme is called 'random rain' and has been shown to give good results for the simulation of crystal growth from solution\textsuperscript{15}. This is the probability scheme used for all growth simulations in this paper, as the Venlafaxine crystals are obtained from a heptane solution. Growth rates can be computed directly from the Monte Carlo results, allowing for a kinetic Wulff construction of the growth morphology.
5.4 Computational methods

5.4.1 Calculation of the crystal graphs

The crystal graphs were calculated using crystal structures obtained from single crystal X-ray diffraction. The geometries of the molecular conformations from the experimental structures were optimized using Gaussian 94 with an HF/6-31G* basis set. A self-consistent field (SCF) convergence criterion of $10^{-8}$ as well as a "Tight" minimization threshold was applied. Charges were fitted to the nuclei using a restricted electrostatic potential (RESP) charge fitting scheme. This RESP procedure serves two goals: first of all, it is known that RESP charges are less sensitive to small perturbations in the geometry, that will happen when the crystal structure is minimized (vide infra). Secondly, the RESP procedure was applied to all polymorph conformers at the same time, in order to obtain a single charge set that describes all molecular electrostatic potentials. In this way lattice energies of the two polymorphs can be compared directly, instead of having to calculate, estimate or ignore the cost in energy for changing the charge distribution going from one conformation to the other. This procedure is based on the R.E.D. v1.0 procedure developed by Pigache et al.

The molecular structures thus obtained were used to build the crystal structures, which were consecutively minimized using the Dreiding force field and the "Smart Minimizer" of Cerius², with Ewald sums for the van der Waals and Coulomb contributions and high convergence settings.

Pairwise interactions were calculated using the Dreiding forcefield in the Cerius² program using a direct calculation of van der Waals and Coulomb contributions with a constant $\varepsilon_r$ value. In contrast, the default value of $\varepsilon_r$ in the Cerius² implementation of the Dreiding forcefield scales linearly with the distance, to approximate solvent effects. Crystal graphs were created from all pairwise interactions up to a certain cutoff radius. The resulting interactions were then scaled so that their sum was equal to the dissolution enthalpy in heptane of each polymorph. Any scaled interactions with bond strength below a certain cutoff-value (usually $kT$) were discarded.

5.4.2 Attachment energy calculations

Once the energies of the bonds making up the crystal graph have been determined, attachment energies can be calculated from the crystal graph by the program Facelift. This program finds all PBCs in the graph and subsequently combines them to form connected nets. The bonds that make up the connected net are the slice bonds; the bonds that attach connected
nets to each other are the attachment energy bonds. The Facelift routine is also implemented in the Hartman-Perdok module of Cerius².

5.4.3 Monte Carlo simulations

The Monty simulations were performed on surfaces \((hkl)\) for which a connected net was found using the Facelift program. All simulations were performed on a rectangular grid of unit cells parallel to the orientation \((hkl)\). For all orientations a simulation box of 50*50 unit cells with lateral periodic boundary conditions was used. The sampling in the simulations was preceded by a period of relaxation, in which the surface was allowed to grow under the same driving force as during the simulation. This relaxation time was taken to be 100000 events in all simulations. The sampling to determine the growth rate was subsequently done at least 500 times, with periods of 10000 events in between. When there was reason to believe that the simulations needed more sampling time, as judged from the development of height vs. number of events, the sampling time was increased to a maximum of 10000 sampling events all with 10000 moves in between. In order to prevent long equilibration times at low driving forces, the simulations were performed on the same surface for decreasing driving force, i.e. the final surface configurations at higher \(\Delta \mu / kT\) were used as the initial surface configuration for simulations at lower \(\Delta \mu / kT\).

5.5 Results and discussion

5.5.1 Crystal graph calculation

Using the methods outlined above, the energies of the crystal structures were minimized using a single charge set for all conformers of the three polymorphs. The resulting lattice parameters and lattice energies are listed in Table 1. The lattice energies are very close, and given the error associated with the force field, no stability ranking can be made on the basis of these energies. The similarity of the crystal structures, as shown in Figure 2, is reflected in the fact that these lattice energies are so close. Next, all pairwise interactions were calculated. For all phases the unscaled interactions below \(-1.5\) kcal/mol were used. The included bonds were subsequently scaled to the dissolution enthalpy.

This procedure resulted in crystal graphs in which the total number of bonds between the growth units is equal to 11 for phase I and 12 for phases II and III. The bonds not related by symmetry are listed in Table 2. It must be noted that for phase II the maximum number of bonds
listed in this Table does not correspond to the total number of bonds between growth units, as some bonds are not present for all growth units. This is due to the fact that for this phase, the number of growth units in the asymmetric unit cell, \( Z' \) is equal to two.

**Table 1: Lattice parameters and energies of the three phases before and after geometry optimization**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Spacegroup</th>
<th>( Z )</th>
<th>( a ) (Å)</th>
<th>( b ) (Å)</th>
<th>( c ) (Å)</th>
<th>( \beta )</th>
<th>Lattice energy kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>P 21/n</td>
<td>4</td>
<td>8.21</td>
<td>8.86</td>
<td>21.79</td>
<td>92.79</td>
<td>-28.6</td>
</tr>
<tr>
<td>-optimized</td>
<td></td>
<td></td>
<td>8.28</td>
<td>9.32</td>
<td>22.47</td>
<td>93.71</td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td>P 21/c</td>
<td>8</td>
<td>8.84</td>
<td>8.27</td>
<td>43.75</td>
<td>90.97</td>
<td>-28.9</td>
</tr>
<tr>
<td>-optimized</td>
<td></td>
<td></td>
<td>9.28</td>
<td>8.35</td>
<td>44.63</td>
<td>92.41</td>
<td></td>
</tr>
<tr>
<td>Phase III</td>
<td>P 212121</td>
<td>4</td>
<td>8.22</td>
<td>8.86</td>
<td>22.27</td>
<td>90.00</td>
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<td></td>
<td>8.25</td>
<td>9.27</td>
<td>22.74</td>
<td>90.00</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Crystal graph bonds for the three phases of Venlafaxine. In the first column the bond offset is listed, in the second the bond strength in kcal/mol. For example, the first line indicates a bond going from GU 1 to GU 2 in the same unit cell. This bond has an unscaled strength of -7.81 kcal/mol and a scaled bond strength of -3.20 kcal/mol. Bonds that are related by symmetry are not listed.**

<table>
<thead>
<tr>
<th>Phase I Bond</th>
<th>Unscaled</th>
<th>Scaled</th>
<th>Phase II Bond</th>
<th>Unscaled</th>
<th>Scaled</th>
<th>Phase III Bond</th>
<th>Unscaled</th>
<th>Scaled</th>
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</thead>
<tbody>
<tr>
<td>1-2[000]</td>
<td>-7.81</td>
<td>-3.20</td>
<td>1-8[100]</td>
<td>-8.13</td>
<td>-5.44</td>
<td>1-3[000]</td>
<td>-7.59</td>
<td>-4.02</td>
</tr>
<tr>
<td>1-3[010]</td>
<td>-5.98</td>
<td>-2.45</td>
<td>1-8[000]</td>
<td>-7.58</td>
<td>-5.07</td>
<td>1-3[100]</td>
<td>-4.55</td>
<td>-2.41</td>
</tr>
<tr>
<td>1-3[000]</td>
<td>-3.98</td>
<td>-1.63</td>
<td>1-5[00-1]</td>
<td>-4.78</td>
<td>-3.19</td>
<td>1-1[-100]</td>
<td>-3.50</td>
<td>-1.85</td>
</tr>
<tr>
<td>1-1[-100]</td>
<td>-3.58</td>
<td>-1.47</td>
<td>1-8[110]</td>
<td>-4.54</td>
<td>-3.04</td>
<td>1-1[0-10]</td>
<td>-2.70</td>
<td>-1.43</td>
</tr>
<tr>
<td>1-1[0-10]</td>
<td>-2.58</td>
<td>-1.05</td>
<td>1-8[010]</td>
<td>-4.52</td>
<td>-3.02</td>
<td>1-4[001]</td>
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<td>-1.42</td>
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<td>-3.49</td>
<td>-2.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                |          |        |               |          |        |                |          |        |
|                |          |        | 1-1[-100]     | -2.54    | -1.70  |                |          |        |
|                |          |        | 1-5[0-1-1]    | -1.60    | -1.07  |                |          |        |
|                |          |        | 2-4[0-10]     | -4.00    | -2.67  |                |          |        |
|                |          |        | 2-2[0-10]     | -3.20    | -2.14  |                |          |        |
|                |          |        | 2-4[-1-10]    | -2.75    | -1.84  |                |          |        |
|                |          |        | 2-2[-100]     | -2.72    | -1.82  |                |          |        |
5.5.2 Experimentally observed habits

Phase I exhibits two distinct morphologies, both displayed in Figure 3. The \((hkl)\) indices were determined using an optical goniometer. Both morphologies have the faces \{002\}, \{10-1\} and \{012\}, one has \{002\} as the largest faces (Figure 3(a)), the other has \{10-1\} as the largest faces (Figure 3(b)). The difference between the two habits is attributed to the presence of a dislocation on the \{002\} face leading to a spiral growth mechanism for the crystals shown in Figure 3(b). The presence of the typical spiral growth pattern on the \{002\} surface was confirmed experimentally using Differential Interference Contrast Microscopy (DICM) for the crystals having the morphology of Figure 3(b) and no spirals were found on the \{002\} surface of the crystals having the morphology displayed in Figure 3(a). These microscopy images are displayed in Figure 4.

![Figure 3](image)

\(\text{Figure 3: Experimental habits of phase I grown from heptane solution. Figure 3(a) shows the rectangular morphology without a spiral pattern on \{002\}, Figure 3(b) shows the morphology with a spiral growth mechanism on \{002\}, leading to a more plank-like morphology with relatively large \{-101\} faces.}\)

The indices of the experimental habit of phase II could not be determined using the optical goniometer, so the experimental habit, shown in Figure 5, has no indices. Still, it can be seen that also this phase has a plank-like shape, with similar top faces as phase I. The crystals of phase II consist of domains parallel to the basal face, which is, very likely, \{002\} \((\text{vide infra})\). These domains were found using a polarization microscope as alternating extinct and bright striped patterns on the side faces, depending on the orientation of the crossed polarizers. These domains were interpreted as twin domains typical for the monoclinic spacegroup P 2\(_1/c\) of this phase in combination with its layered crystal structure (see Figure 2). As a result we can conclude that the basal face is \{002\} and the \(b\)-axis is parallel to the long axis of the crystals. The presence of
these domains and the corresponding domain walls gives rise to a lot of macrosteps and striations on the side and top faces, as can be seen in the lower image of Figure 5. Such striations can act as sources for growth steps leading to vicinal orientations. This explains the problem with indexing the habit of phase II.

The experimental habit of phase III is also a flat plank-like shape, displayed in Figure 6. The indices are \{002\} (the basal face), \{101\} (side faces) and \{012\} (top face).

![Figure 4: Differential interference contrast microscopy (DICM) images of phase I shows (a) the presence of 2D islands and (b) spiral growth hillocks. The magnification factor is 200x. These micrographs were taken of the \{002\} surface for samples corresponding to the two different morphologies as displayed in Figure 3.](image)

![Figure 5: Experimental habit of phase II grown from heptane solution. This habit could not be indexed on a goniometer, so no indices are given.](image)
5.5.3 Prediction of morphologies

5.5.3.1 BFDH

The morphologies were first predicted using the BFDH theory. The results for the three phases of Venlafaxine are displayed in Figure 7.

As can be seen in these figures, the predicted morphologies of phase I and III are very similar, due to the fact that the lattice parameters are quite similar. Differences are mainly caused by the difference in spacegroup, $P2_1/c$ for phase I and $P2_12_12_1$ for phase III. As the unit cell of phase II has a $c$-axis that is about twice as long as the $c$-axes of phase I and III, the predicted shape of phase II is more plate-like. None of the predictions of the BDFH theory are very good however, as square shapes are predicted, instead of the experimentally observed plank-like...
morphologies. Also, for phase I and III, the predicted shapes are too thick in the \( \{002\} \) orientations.

### 5.5.3.2 Attachment energies

For phase I the Facelift procedure results in a set of 170 PBCs and 63 connected nets in 13 crystallographic forms; for phase II however, these numbers are much higher because of the larger number of particles in the unit cell. For phase II 36922 PBCs and 4427 connected nets in 25 crystallographic forms were found. For phase III, 288 PBCs were found which could be combined to form 57 connected nets in 9 crystallographic forms.

The connected net attachment energies of phases I, II and III are shown in Table 3.

*Table 3: F-faces and corresponding attachment energies of the three phases of Venlafaxine.*

<table>
<thead>
<tr>
<th>Phase I ((hkl))</th>
<th>Phase II ((hkl))</th>
<th>Phase III ((hkl))</th>
</tr>
</thead>
<tbody>
<tr>
<td>({002})</td>
<td>-23.25</td>
<td>({00-2})</td>
</tr>
<tr>
<td>(-101)</td>
<td>-36.21</td>
<td>({0-1-1})</td>
</tr>
<tr>
<td>({01-1})</td>
<td>-46.50</td>
<td>({012})</td>
</tr>
<tr>
<td>({01-2})</td>
<td>-46.83</td>
<td>({10-4})</td>
</tr>
<tr>
<td>(-10-1)</td>
<td>-48.88</td>
<td>({013})</td>
</tr>
<tr>
<td>({110})</td>
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- 118 -
The predicted morphologies obtained from these data are shown in Figure 8. This prediction is much better than that of the BFDH method; for phase I, the \{002\} and \{10-1\} orientations are both predicted correctly. The attachment energy of the \{011\} orientation is very close to that of \{012\} orientation, so the former is favored over the latter in the predicted morphology. The observed rectangular plank-like is not reproduced, however, as the predicted morphology is almost square. Phase II also has an almost square attachment energy morphology, and orientations \{002\}, \{100\}, \{-102\} and \{011\} are predicted. The third phase has the orientations \{002\}, \{101\} and \{011\} correctly predicted, and \{110\} predicted but not observed. The general shape is too thick and square, as opposed to the elongated plank-like shape of form III, observed experimentally.

Figure 8: The Attachment energy morphologies of the three phases of Venlafaxine.
5.5.3.3 Monte Carlo growth simulations

The growth rates of all connected net orientations of phase I as a function of the relative driving force $\Delta\mu / kT$ are displayed in Figure 9.

![Figure 9: Monte Carlo growth rate simulations for phase I.](image)

As can be seen from the figure the three experimentally observed orientations have the lowest growth rate in the right order $R_{\{002\}} < R_{\{10-1\}} < R_{\{012\}}$. The habit at a relative driving force of $\Delta\mu / kT = 2.4$, the lowest value simulated where the $\{002\}$ form shows growth, is displayed in Figure 10.

![Figure 10: Predicted morphology for phase I at $\Delta\mu / kT = 2.4$.](image)

The existence of two distinct habits in one single crystallization batch (see Figure 3) can be explained by the fact that the $\{002\}$ orientation grows either via a spiral growth mechanism or via a 2D nucleation mechanism. To test this hypothesis, simulations of the $\{002\}$ orientation with a spiral growth mechanism were also performed. This is accomplished by using a dislocation line bounded by two dislocations with opposite Burger's vectors (see also Figure 11). The height of the Burger's vector is equal to the length of the c-axis.
The resulting growth morphology of phase I, also at a relative driving force of 2.4, with the \{002\} form having a spiral growth mechanism, is displayed in Figure 12. The morphological importance of the \{002\} face is much lower than without a spiral growth mechanism (see Figure 10) and the \{10-1\} face becomes dominant, as was also seen experimentally.

For phase II, the simulated growth rate as a function of $\frac{\Delta \mu}{kT}$ is displayed in Figure 13. At $\frac{\Delta \mu}{kT} > 7.0$, the \{002\} orientation starts to grow. The morphology at that value for $\frac{\Delta \mu}{kT}$ displayed in Figure 14. As can be seen from this figure, when comparing it to the experimental morphology, the predicted morphology is not correct. An octagonal flat crystal is predicted, with the large face being \{002\}, and the side faces \{10-4\}, \{110\} and \{011\}. Either $R_{10-4}$ or $R_{011}$ is too large, resulting not in a plank-like morphology, but in this octagonal shape.
The high driving force at which phase II is predicted to start growing, i.e. $\mu/kT > 7.0$, hints at the fact that another growth mechanism may be dominant on the {002} orientation. To see the effect of spiral growth, simulations were run using a spiral growth mechanism. Using the spiral growth mechanism for phase II a more plank-like morphology is predicted at lower driving force value, $\mu/kT = 4.0$ (see Figure 15). This shape corresponds better to the experimentally observed morphology. Comparing, however, the orientation of the top faces with the experimentally observed ones (see Figure 5); the prediction seems to be incorrect. As discussed before, the presence of domains in this phase leading to striations on the top and side faces probably gives rise to ill-defined orientations, which could not be indexed. The growth of these faces is then determined mainly by the domain boundaries acting as step sources, rather than by a 2D nucleation mechanism. Such an effect of domain walls has been observed for, e.g., gibbsite crystals\textsuperscript{22}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure13}
\caption{Monte Carlo growth rate simulations for phase II.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure14}
\caption{Predicted morphology for phase II at $\mu/kT = 7.0$.}
\end{figure}
Figure 15: Predicted morphology at $\Delta \mu / kT = 4.0$ for phase II with the {002} orientation having a spiral growth mechanism. The side faces are {104} and the top faces are {014}.

The simulated growth rates of phase III are displayed in Figure 16 and the predicted morphology at $\Delta \mu / kT = 4.0$ is displayed in Figure 17.

Figure 16: Monte Carlo growth rate simulations for phase III.

Figure 17: Predicted morphology for phase III at $\Delta \mu / kT = 4.0$.

Although the predicted morphology is quite close to the experimentally observed habit, the predicted indices of the side faces are not correct. The short edges of the rectangle are predicted
to be \{101\}, but actually they are \{012\}, and vice versa. As phase III grows as a conglomerate of stacked domains of both enantiomorphs\textsuperscript{12} the relative morphological importance of these two faces might be influenced by that. Face indexation on a goniometer shows that both the conglomerate and the enantiopure crystals have the same indices and morphological importance, in contrast to phase II. This is explained by the difference in space group symmetry for the phases: phase II has a monoclinic space group (P 2\(_1\)/c) with its unique axis parallel to the long axis of the crystal. This gives rise to mutually inclined microfacets on the top faces for the twin domains. This, together with the domain boundaries acting as step sources explains the inability to index the crystals of phase II. Phase III, on the other hand, has an orthorhombic space group (P2\(_1\)2\(_1\)2\(_1\)) leading to no inclination between the microfacets on the top faces. The presence of the domains, however, can still lead to different growth rates for the top faces, as well as the side faces, compared to the predictions.

### 5.6 Conclusions

From the free base form of the anti-depressive drug Venlafaxine, the dominant morphologies of three polymorphs were studied experimentally. Using theories with different levels of complexity, the observed growth morphologies were correlated with the predicted morphologies. For all polymorphs the predicted morphology according to the BFDH theory does not give good results. The Hartman-Perdok theory does better, but fails to predict some indices and - more importantly - fails to predict the rectangular plank-like habits of the polymorphs and instead predicts square plank or cube-like morphology.

The results of the Monte Carlo growth simulations, which account for both the driving force and the growth mechanism, are very promising. For phase I the predictions reproduce the experimentally observed morphology using a 2D nucleation mechanism. A second habit, also observed experimentally, in which \{-101\} is the dominant face, is found for phase I when a spiral growth mechanism is used for the \{002\} orientation.

The simulated growth rate of the \{002\} orientations of phase II was very low until high driving forces. Consequently, the predicted morphology did not agree very well with the experimentally observed morphology. When a spiral growth mechanism was used here, the morphology could be predicted at lower \(\triangle \mu /kT\) values. This reproduced the experimentally observed morphology more accurately. The observed domains in the crystal structure of phase II, interpreted as twin domains, may explain the differences between the morphologies obtained
using simulations of 2D nucleation and the observed morphology. The twin domains may act as step sources, facilitating growth at lower driving force.

The simulations of phase III also show a rectangular plate-like habit, in close correlation with the observed habit. The relative morphological importance of the \{101\} and \{012\} faces is not reproduced however; the \{101\} faces are predicted to be at the short end of the rectangle, whereas the \{012\} faces are experimentally observed to be the short side of the rectangle, and vice versa.

It can thus be concluded that the Monty simulations are a promising tool for studying crystal growth. Although not all indices of phase III could be predicted correctly, all phases did give a semi-quantitative agreement between the experimentally observed habit and the predicted morphology, as far as the overall shape is concerned. The inability of the BFDH method and attachment energy method to predict the morphology correctly for any of the phases shows that the Monte Carlo simulations are a welcome addition to the set of tools for morphology prediction. It is not surprising that when crystals show a more complicated growth behavior than that captured by the mechanisms used by Monty, e.g. when twinning causes striations and macrosteps, which in turn act as step sources, the simulations no longer show a good agreement with the observed growth behavior.

Acknowledgements

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5.7 References

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Chapter 6
ONDANSETRON FREE BASE: POLYMORPHISM OR ORDERED DISORDER?

Abstract

For Ondansetron free base, an active pharmaceutical ingredient that is used to inhibit vomiting and nausea during chemotherapy, two solid forms grown from solution have been claimed as polymorphic forms. Characterization using X-ray powder diffraction, DSC and solid state NMR, however, turns out to be insufficient to decide if one deals with polymorphism. From the combination of a badly resolved crystal structure of a vapour grown single crystal of a third form and molecular modelling we concluded that these forms are best interpreted as locally ordered solid solutions of enantiomers. Different local occupations of enantiomers, induced by the amount of water present during crystal growth, give rise to the slightly different forms of Ondansetron free base. The results of the present study show that subtle but significant differences in X-ray powder diffraction do not automatically imply polymorphism.
6.1 Introduction

Many pharmaceutical active ingredients can crystallize in more than one crystal structure. This phenomenon is called polymorphism. McCrone defines a polymorph as ‘a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state’¹. The amorphous state is sometimes also regarded as a polymorph, but this is not correct. Which polymorph is obtained depends on the conditions during crystallization: solvent, concentration, temperature and pressure. Also the crystallization method can have an effect on the polymorphic outcome, e.g. cooling crystallization, evaporative crystallization, anti-solvent addition and vapour growth. A general overview of analytical methods to examine polymorphs was published by Threlfall².

Polymorphism is of importance in several areas but especially for the pharmaceutical industry³. As polymorphs have different crystal structures they can display different physical properties, in particular they can have a different bio-availability. Because finding a new polymorph with advantageous properties is regarded as an invention, such polymorphs can be patented. It is therefore important to understand what constitutes a polymorph and to know when we are dealing with polymorphism⁴.

Normally it is clear if one is dealing with polymorphism, but there are borderline cases in which this it is not the case. Examples are found in case of mesomerism, tautomerism, faulted layer stacking and other kinds of disorder. Recently it was demonstrated that barbituric acid could be converted mechanically into a mesomeric structure, the trihydroxy form, as was demonstrated using X-ray powder diffraction and solid state NMR⁵. Dissolved, the new phase converts immediately back to the starting configuration. A question arises whether this is polymorphism or a matter of different compounds in the solid state. In case of tautomers (molecules with different electronic structures), one can ask the question if tautomers are the same or different molecules or compounds. A recent example is encountered in the drug omeprazole for which 5 different single crystals could be obtained that contain variable amounts of the two tautomers, the 5- and the 6 –metoxy derivative⁶. The crystal packing of both tautomers is the same and any crystal can be modelled as a solid solution of the two tautomers. Another borderline example, concerning faulted layer stacking, is Aspirin. Recently a second polymorph of Aspirin was claimed⁷. Both forms are composed of almost identical layers of Aspirin dimers. The distinction between the two forms is the way the layers are stacked. According to Desiraju the newly claimed form of Aspirin is not adequately described by the
form II structure alone, but it is better regarded as intergrowth of structural domains of the two forms of stacked layers\textsuperscript{8,9,10}. Different form II crystals contain different amounts of the two domains which implies that an infinite number of Aspirin polymorphs would exist. Very recently it was shown that different X-ray diffraction patterns of Eniluracil samples could be better rationalized by disorder than by polymorphism\textsuperscript{11}. From these examples it is clear that the distinction between polymorphism and other possible structural differences found for many organic molecules is not always straightforward. Because of patent protection of different solid forms of the same compound, this has also legal implications.

In the present study the structural relation between two claimed polymorphs, ODS-1 and ODS-2, of solution grown Ondansetron free base\textsuperscript{12,13}, and a vapour grown third form is investigated. Ondansetron is on the market as the dihydrate of the hydrochloric acid salt. It is a member of the therapeutic class of antiemetic drugs and it is used to inhibit vomiting and nausea by decreasing the gastric motility, mainly during cancer chemotherapy and radiotherapy and post-operative states. The compound has one chiral centre and is on the market as a racemate. The official IUPAC name is: (±)1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one. For the chemical structure see figure 1.

\[ \text{Figure 1: Molecular structure of Ondansetron and carbon numbering used.} \]

From the results of the present study we propose that the structure of Ondansetron free base is a locally ordered solid solution of enantiomers, and that the differences in the X-ray diffraction patterns (XRPD) can be explained by small differences in the structure on a mesoscopic scale, possibly related to a small difference in water content present during crystal growth.
6.2 Experimental Procedures

6.2.1 Sample preparation

Racemic Ondansetron with a purity of 99.9%, according to High Pressure Liquid Chromatography (HPLC), was supplied by Synthon BV. The solvents used (pa) were purchased from Aldrich and were used without further purification.

Two samples were prepared via crystallization from solution. For the preparation of ODS-1 the following procedure was used: 0.5 g of Ondansetron was dissolved in a mixture of 25 ml of ethanol and 10 ml of water while heating. The clear solution was cooled to room temperature and left at that temperature for a few days. The formed crystals were filtered off and dried in air for 2 days. Solution NMR showed that no ethanol was present in the sample. For the preparation of ODS-2, the following procedure was used: 0.3 g of Ondansetron was dissolved in 50 ml of methanol while heating. The clear solution was cooled to room temperature and left at that temperature for a few days. The formed crystals were filtered off and dried in air for 2 days. Solution NMR showed that no methanol was present in the sample. A third form of Ondansetron, ODS-3, was prepared in single crystalline form using vapour growth, at a temperature of 433 K and a pressure of 1.9 \times 10^{-4} \text{ bar} in a glass tube. The starting material was placed at the lower end of the glass tube, which was positioned in the hot part of a horizontal furnace. Crystals were deposited at the colder end of the tube.

6.2.2 Sample characterization

Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC 822e apparatus. Nitrogen was used as an inert purge gas. The samples were analyzed using 40 μl aluminium pans with a pierced lid. The heating rates used were: 0.1, 1 and 10 °C/min.

Thermo gravimetric analysis (TGA) analyses were performed using a Mettler Toledo TGA/SDTA851e analyser. Nitrogen was used as an inert purge gas. The samples were analyzed using 40 μl aluminium pans with a pierced lid and the heating rate was 10 °C/min.

Karl Fisher measurements were performed using a Mettler Toledo DL39 coulometer or a Mettler Toledo DL 58 titrator.

Optical microscopy images were made using a Zeiss Axioplan2 microscope. For that the samples were suspended in oil.

XRPD was performed using a Bruker D8 Advance X-ray diffractometer equipped with a VÅNTEC detector. The diffractometer was equipped with a Johansson type monochromator. The data were collected in reflection, transmission or capillary mode, using monochromatic Cu
Kα1 radiation. The X-ray diffraction experiments in a climate chamber were performed using a heating rate of 1 °C/min, while keeping the temperature constant during 10 minutes after each heating step of 10 °C. Upon heating, the sample was flushed with dry nitrogen; upon cooling with ambient air.

For the single-crystal X-ray diffraction experiments, a single crystal, dimensions 0.19 x 0.05 x 0.03 mm³ was mounted in air on a glass fibre. Intensity data were collected at a temperature of -65°C. A Nonius Kappa CCD single-crystal diffractometer was used (φ and ω scan mode) using graphite monochromated Mo-Kα radiation. Unit cell dimensions were determined from the angular setting of 17 reflections. Intensity data were corrected for Lorentz and polarization effects. For absorption correction the Siemens Area Detector ABSorption correction program (SADABS) was applied. The structure of ODS-3 was solved using the program CRUNCH and was refined with standard methods using SHELXL with anisotropic parameters for the non-hydrogen atoms. The hydrogens were placed at calculated positions and were refined riding on the parent atoms.

Solution NMR was performed using an Avance 400 instrument with DMSO-d₆ as solvent.

Cross Polarization Magic Angle Spinning (CPMAS) ¹³C solid state NMR spectra were acquired on a 400 MHz Chemagnetics Infinity spectrometer. A 4 mm and a 3.2 mm double resonant probe were used, tuned to 100.58 MHz for carbon and 399.95 MHz for protons. Measurements on ODS-1 and ODS-2 were done on the 4 mm probe with 8.0 kHz MAS. Here variable amplitude cross polarisation (VACP) with a contact time of 2 ms was used with a radio frequency (RF) field strength of 64 kHz on protons and 56 kHz on carbon and a +1 to -1 kHz linear ramp on protons. During acquisition the protons were decoupled with a two pulse phase modulation (TPPM) pulse sequence with a 110 kHz RF field, a pulse duration of 5.3 μs and a phase modulation of 15 degrees. For proton decoupling the continuous modulation (CM) scheme was used with a modulation amplitude of 0.12 radians, a period of 8.4 μs and an RF field strength of 110 kHz. Peak intensities were obtained by deconvolution of the spectra using a Voigt lineshape. The data were processed using the MatNMR processing package which runs under Matlab.

Molecular mechanics modelling was performed using the Dreiding v2.21 force field, as implemented in the Cerius² modelling environment. Atomic partial charges were generated using the Gasteiger charge equilibrium method. The geometry of the crystal structures was optimized using Ewald summation for the van der Waals and electrostatic interactions. A constant value of εᵣ was used in the electrostatic calculations. Energies of individual molecules
were calculated without a cut-off distance for van der Waals and electrostatic interactions. Lattice energies were calculated by subtracting the total energy of the individual molecules from the total energy of the crystal structure.

6.3 Results and Discussion

In order to determine the structure differences between the two solution grown forms of Ondansetron free base, the characterization of the two samples was started using optical microscopy. These observations showed that both samples, ODS-1 and ODS-2, consist of needle-like crystals, see figure 2.

![Optical microscopy images of ODS-1 and ODS-2 using oil immersion.](image)

*Figure 2: Optical microscopy images of ODS-1 and ODS-2 using oil immersion.*

In figure 3 the XRPD patterns of the samples crystallized from an ethanol/water mixture, ODS-1, and crystallized from methanol, ODS-2 are presented. Although the two XRPD patterns are very similar, they show significant peak shifts and intensity differences. This is an indication for polymorphism. TGA and Karl-Fisher measurements showed a mass loss of 1.8 % for ODS-1 and of 1.6 % for ODS-2. As a first check to find the influence of the water on the crystal structure, both samples were subjected to a climate chamber experiment, in which temperature and humidity can be controlled separately. The samples were heated to 155 °C, during which the samples were expected to dehydrate completely, and possibly become more similar. Interestingly however, the difference between the XRPD patterns of the two “anhydrate” samples at elevated temperatures (figure 4) increases. Upon cooling under ambient conditions, however, both patterns reverted back to their original “hydrated” pattern.
Figure 3: XRPD patterns of samples of ODS-1 and ODS-2, including a difference plot. The difference plot is shifted downwards for clarity to prevent overlap of difference and XRPD patterns.

Figure 4: XRPD patterns of samples of ODS-1 and ODS-2 heated to 155 °C, including a difference plot. The difference plot is shifted downwards for clarity.

Because the XRPD patterns do not yield a clear picture, DSC thermograms were recorded for ODS-1 and ODS-2 using different heating rates, see figure 5. Using a heating rate of 10 °C/min the temperature difference in the onset of the melting endotherm for the two samples is small; 246 °C in case of ODS-1 and 245 °C in case of ODS-2. The melting endotherm is followed
in both samples by a degradation endotherm, as was confirmed using solution NMR. Using a heating rate of 1 °C/min the onset temperature of the melting endotherms has the same value for both samples, and also in this case the melting endotherm is followed by a degradation endotherm, although more clearly visible for ODS-1. Lowering the heating rate to 0.1 °C/min results in two different thermograms; the melting endotherm is split into a doublet in case of ODS-1, while for ODS-2 the melting endotherm is still a singlet. The onset temperature of the first endotherm for ODS-1 is almost the same as the onset temperature for ODS-2; 215 versus 217 °C. The second endotherm of the ODS-1 sample is a degradation endotherm, which could point at a less stabilizing surrounding of the molecules in ODS-1 crystals. In all thermograms a thermal event between 50 and 150 °C is visible. This can be attributed to the loss of water. As can be expected, the water loss takes place at lower values when using lower heating rates, the same is valid for the onset temperatures of the melting and degradation endotherms.

![DSC thermograms of ODS-1 (left panel) and ODS-2 (right panel) recorded with different heating rates.](image)

Figure 5: DSC thermograms of ODS-1 (left panel) and ODS-2 (right panel) recorded with different heating rates.

To obtain an impression of the mutual stability of the two crystalline phases, slurry experiments were performed. Judging from the XRPD patterns, ODS-1 could be transformed to ODS-2, by stirring the sample for 3 days at room temperature in methanol. Conversely stirring ODS-2 in a 2.5/1 mixture of ethanol/water for 3 days resulted in the formation of ODS-1. These results indicate that ODS-1 and ODS-2 are not real polymorphs, but that perhaps different levels of water have incorporated.

Several attempts were made to grow single crystals of Ondansetron free base, but because of the high nucleation barrier and the extreme fast growth in the needle direction it was not possible to obtain single crystals that were large enough to perform a single crystal structure determination. Using vapour growth, however, small single crystals of Ondansetron could be obtained. The resulting crystal structure is presented in figure 6, for crystal data see table 1.
Initial refinement showed exceptional large and anisotropic thermal ellipsoids for carbon atoms 2 and 3 (in both molecules in the asymmetric unit) together with a bad geometry for these atoms. This indicated that these atoms are disordered over two positions which correspond to two possible conformers of the Ondansetron molecules. A suitable model describing this disorder could be defined and refinement in spacegroup \( P-1 \) \((Z = 4)\) led to acceptable geometries for the resulting positions of atoms 2 and 3. Refinement of the occupancy factors for the possible conformers of the Ondansetron molecules showed that for both symmetry independent molecules the two conformers are present in almost equal amount. This disorder model allows either enantiomer to be present at both independent positions of the asymmetric unit and, at the same time, restricts the possible conformers to combinations of a particular enantiomer with one of the two conformations of the six-membered ring. In case of the \( R \)-enantiomer the six-membered ring is up, compared to the plane through the indole part of the Ondansetron molecule, in case of the \( S \)-enantiomer the six-membered ring is down, see figure 6.

![Figure 6: One of the two Ondansetron molecules in the asymmetric unit of ODS-3.](image)

The carbons 2 and 3 are disordered over two positions, indicated by the dashed lines, corresponding with the six-membered ring “up” or “down” compared to the plane through the indole part of the molecule. Note, that these two ring orientations correspond to different enantiomers.

The (2-methyl-1H-imidazole-1-yl)-methyl sidegroup of one of the Ondansetron molecules is badly defined by the data and could only be refined by restraining its geometry to the corresponding sidegroup of the other molecule. Apparently, the disorder in the conformation in the six-membered ring, formed by atoms 1,2,3,4,4a and 9a, also influences the orientation of this sidegroup. From a difference Fourier map a water molecule was found with an occupancy factor
of around 50%. The water molecule was found to bridge the imidazole side groups of the Ondansetron in the solid state. As the sample was obtained using growth from the vapour phase, at low pressure and high temperature, it is very unlikely that this water was present during the crystal formation. The presence of the water in the structure was confirmed using Karl-Fisher titrations and determined to be approximately one molecule of water per unit cell, which is in accordance with the occupancy of 50%. Comparing the XRPD pattern, calculated from the crystal structure, with the measured XRPD patterns of ODS-1 and ODS-2 revealed that the calculated pattern shows large similarity with the pattern of ODS-2, that is the crystals grown in a water poor environment.

It is important to realize that in solution both enantiomers are present in the two possible ring conformations. These four possibilities are presented in figure 7 and labelled ‘R-up’, ‘R-down’, ‘S-up’, and ‘S-down’. These conformers could be responsible for the structural differences of ODS-1, or even ODS-2, with respect to ODS-3.

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Table 1: Crystal data of ODS-3.
To get a better insight in the possible conformations of the Ondansetron molecules in the solid state, molecular modeling was performed. Because the XRPD patterns of the three forms are too close to allow for truly different packings, as a starting point the (badly resolved) single-crystal X-ray structure from the vapour grown crystal was taken, but now allowing all four conformations to occur. This structure was refined in space group P-1, i.e. having an inversion centre. The four conformations of the two molecules in the asymmetric unit then lead to a total of 16 different possible unit cell structures. It should be mentioned, however, that the molecular modelling was performed without incorporating water in the crystal structure.

For the four conformations of figure 7, a starting configuration was determined from a conformer search in which the two dihedral angles of the bonds between carbons 3 and 1" and between carbon 1" and the imidazole nitrogen were varied systematically. Each generated conformer was minimized, thus yielding the combination of the two dihedral angles having the lowest energy. These four starting conformers were used two at a time to generate the asymmetric unit of the crystal structure, by aligning the well-resolved conjugated indole ring system from the single X-ray-structure with the molecules generated from the conformer search. After rebuilding the crystals the lattice energy was minimized. This last minimization was performed in a two-step procedure: first the newly placed molecules were allowed to find their minimum energy geometry in the crystal structure without changing the cell parameters and in a second minimization run, the cell parameters were allowed to relax too. This was done to
prevent large changes in the cell parameters upon minimization of the newly placed molecules in the crystal, which might have unfavourable intermolecular interactions due to their initial conformations. From the building of the crystals and the subsequent minimization, it was found that only combinations of the R enantiomer having the ‘up’ ring-puckering and the S enantiomer having the ‘down’ puckering led to crystal structures in which 1) crystal structures could be obtained without molecules overlapping, 2) the ring puckering was left unchanged upon minimization and 3) the lattice parameters did not change much compared to the known lattice parameters. This implies that for all three forms of ODS, these are the only possible combinations. As can be seen from table 2, four possible crystal structures are found when the R-up molecules and S-down molecules are positioned in any combination, without significant differences in lattice energy: the four lattice energies are within 1 kcal/mol, which is within the error associated with the force field used. This indicates that there is no combination that is strongly favoured energetically. This then leads to the hypothesis that the structure of Ondansetron, ODS-3, is in fact a solid solution-like packing of enantiomers.

### Table 2: The four fillings of the asymmetric unit for Ondansetron with the lowest and comparable lattice energies after minimization.

<table>
<thead>
<tr>
<th>Molecule 1</th>
<th>Molecule 2</th>
<th>Lattice energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R up</td>
<td>R-up</td>
<td>-33.13</td>
</tr>
<tr>
<td>R-up</td>
<td>S-down</td>
<td>-33.57</td>
</tr>
<tr>
<td>S-down</td>
<td>R-up</td>
<td>-33.01</td>
</tr>
<tr>
<td>S-down</td>
<td>S-down</td>
<td>-33.69</td>
</tr>
</tbody>
</table>

The obtained results of the molecular modelling were used to further refine the crystal structure of ODS-3. One of the four possible crystal structures of ODS-3 is presented in figure 8. Calculated XRPD patterns of ODS-3 and of the four possible crystal structures are presented in figure 9. Because only very small peak shifts and intensity differences can be observed between the calculated XRPD patterns, this supports the hypothesis that the structure of ODS-3 is a solid solution-like packing of enantiomers.
Figure 8. One of the four possible sequences for the R and S enantiomers for centrosymmetric cases. In this case positions 1 and 2 are occupied by R molecules and positions 1’ and 2’ by S molecules.

Figure 9. Calculated XRPD patterns of ODS3 and of the four possible crystal structures. The labeling of the traces refers to occupation of sites 1, 2, 1’ and 2’ (cf. figure 8) by S and R molecules.

Such solid solution behaviour should be apparent using ss NMR spectroscopy. Therefore ss CPMAS C-13 NMR spectra of powders of ODS-1 and ODS-2 were recorded. The C-13
spectra of both samples are presented in figure 10, showing quite broad peaks. The carbon peak assignments are given in table 3. As can be seen from the spectra and the table there are no big differences in peak positions between the two forms. The signal for carbon 5”, at approximately 13 ppm for both samples, is split in multiple peaks, which means that different surroundings exist for this carbon atom. This observation is also valid for carbon 1, at around 21 ppm, carbon 2 at around 25 ppm and carbon 1” at around 45 ppm. The results of the solid state NMR experiments confirm the solid solution-like packing. From the carbons influenced by the conformation of the 6-membered ring, the 1” and the 5” carbon, in the side group, are mostly affected. Both show three different peaks, which can be associated with three different surroundings of these carbons in the solid state, originating from the four different combinations giving rise to three different types of neighbours: R-up next to R-up, R-up next to S-down and S-down next to S-down.

![Figure 10: ss CPMAS C-13 NMR spectra of ODS-1 and ODS-2.](image)

Considering the resemblance between the XRPD patterns of ODS-3, ODS-2 and ODS-1 it can be argued that all Ondansetron samples have comparable structures. The differences in the XRPD patterns between ODS-1 and ODS-2 can now be explained by differences in mesoscopic structure, as a result of different packing sequences of the two enantiomers, probably induced by different amounts of water present in the structure during crystal growth. ODS-2 and ODS-3 were both grown under “water free” conditions; in case of ODS-2 methanol with only a small amount of water was used, in case of ODS-3 elevated temperatures and high vacuum were used. This is contrary to the case of ODS-1, where a large amount of water was present during crystallization. Once the packing structure is formed it is not altered anymore as was demonstrated by the climate chamber experiments: the de-hydrated forms revert back to the original hydrated forms upon cooling under ambient conditions.
The morphology of the crystals supports the disordered hypothesis. As is shown in figure 2 the crystals obtained from ODS-1 and ODS-2 are needle-like. Growth is fast in the needle direction because of very low barriers for the incorporation of molecules, even at very low supersaturations. When molecules are incorporated that fast into the solid, the likelihood for the occurrence of defects is larger. In this case the ‘defect’ occurs because the lattice does not discriminate between the different enantiomers when they are built in as long as the conformation of the molecule is adapted to conform to the two possibilities, R-up and S-down.

Thus the different forms of Ondansetron are instead of two polymorphs probably two manifestation of a locally ordered solid solution as was demonstrated by the various techniques used.

**Table 3: C-13 NMR peak assignments for ODS-1 and ODS-2.**

<table>
<thead>
<tr>
<th>Carbon</th>
<th>ODS-1 (ppm)</th>
<th>ODS-2 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$5''$</td>
<td>13.25</td>
<td>12.72</td>
</tr>
<tr>
<td>$5'$</td>
<td>14.03</td>
<td>14.00</td>
</tr>
<tr>
<td>$5''$</td>
<td>15.39</td>
<td>15.33</td>
</tr>
<tr>
<td>1</td>
<td>20.76</td>
<td>20.81</td>
</tr>
<tr>
<td>1</td>
<td>21.60</td>
<td>21.58</td>
</tr>
<tr>
<td>2</td>
<td>24.57</td>
<td>24.57</td>
</tr>
<tr>
<td>2</td>
<td>25.48</td>
<td>25.05</td>
</tr>
<tr>
<td>$1'$</td>
<td>28.52</td>
<td>28.42</td>
</tr>
<tr>
<td>$1''$</td>
<td>43.28</td>
<td>43.32</td>
</tr>
<tr>
<td>$1''$</td>
<td>45.37</td>
<td>45.40</td>
</tr>
<tr>
<td>$1''$</td>
<td>48.51</td>
<td>48.48</td>
</tr>
<tr>
<td>3</td>
<td>46.63</td>
<td>46.55</td>
</tr>
</tbody>
</table>

**6.4 Conclusions**

From the combination of all techniques used we conclude that ODS-3 is best interpreted as a locally ordered solid solution of enantiomers; at each site in the structure either an R-up or an S-down Ondansetron molecule can be placed. Because the XRPD patterns of ODS-3 and ODS-2 are very similar, and in turn quite similar to the powder pattern of ODS-1, we assume that also
for ODS-2 and ODS-1 a locally ordered solid solution of enantiomers is present in the solid state. Different combinations of enantiomers in the solid state give rise to slightly different forms of Ondansetron. The amount of water present during the crystal growth induces subtle but important local order in the disorder.

Although the various forms of ODS show different physical properties, one cannot speak of polymorphism. More generally, the results show that subtle but significant differences in X-ray powder diffraction patterns do not automatically imply polymorphism.
6.5 References

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**SUMMARY**

In this thesis different aspects of polymorphism, the ability of a substance to crystallize in more than one crystal form, are studied. Because physical properties, e.g. melting enthalpy, heat capacity, and solubility, of two polymorphs can differ, the pharmaceutical industry spends a lot of money and effort in studying the phenomenon of polymorphism, because especially the solubility difference between two polymorphs can have influence on the effectivity, the bioavailability and the safety of drugs. A lot of effort is invested to find the most stable polymorph, although one is never certain that the most stable polymorph has been found. A recent example, in which the difference in bio-availability was of importance, is ritonavir. After having been on the market for almost two years, a more stable polymorph with a lower solubility and, therefore, a lower bio-availability was found. Especially the thermodynamic aspects, that play an important role concerning polymorphism of active pharmaceutical ingredients of drugs, are studied in this thesis.

After a general introduction about the phenomenon of polymorphism, the thermodynamic theory of solubility of compounds in solvents is described, with the emphasis on compounds exhibiting polymorphism. The relation between phase diagrams and solubility is treated. Starting with simple models describing the thermodynamics of solutions, assuming no mixing between the solvent and the solute in the solid state, the large variety of possible solubility curves, describing the temperature dependent solubility, are explained. In addition, pseudo polymorphs, that is solvates, are treated. The models result in a formula which makes it possible to estimate the transition temperature for an enatiotropically related system. For this only the melting temperature and the melting enthalpy of the two polymorphs are needed. Both are simply determined using Differential Scanning Calorimetry (DSC).

In the third chapter the phase behaviour of the free base of Venlafaxine, the active ingredient of a drug used to treat stress, is described. Both the polymorphic phase diagram as well as the thermodynamic relations between the three forms are studied, using DSC, X-ray Powder Diffraction (XRPD), slurry experiments and solubility measurements. The solubility of Venlafaxine was determined in several solvents, focussing on deviations of the solubility as compared to the ideal solubility according to the van ’t Hoff equation. The nature of the deviation of the solubility was such that attributing the stability regions for polymorphs I and II, on the basis of solubility data would lead to a wrong attribution of these regions. This turned out to be the result of the concave shape of the solubility curves, a form that, from a theoretical
viewpoint, is described in more detail in chapter 2. The transition temperature of polymorph I and II of Venlafaxine free base was calculated using the earlier mentioned equation to estimate the transition temperature and compared with the values extracted from the solubility data. The concave shape of the solubility curves is the result of a non neglectable temperature dependence of the melting enthalpy.

In chapter 4 the remarkable behaviour of the free base of Venlafaxine with respect to polymorphism and chiral resolution is studied. Using different complementary techniques, the three forms of Venlafaxine free base were characterized. The crystals of all the three forms are composed of almost identical enantiomerically pure layers, only the stacking of the layers is different. In case of form I alternating bi-layers of R and S layers were found, while form II consisted of alternating double bi-layers of R and S enantiomers. In case of form III, the form with the highest melting point, the enantiomer separation is complete, resulting in a racemic conglomerate. The racemic conglomerate can be obtained from solution, or via a solid-solid phase transition of the lowest melting form. Remarkably, during this phase transition the shape of the crystal is conserved. It is hypothesized that, during the phase transition occurring, the chiral separation takes place via a local melting process. Locally the melting point is lower as the result of crystal defects. Because of the local melting process, molecules are able to migrate over relatively large distances between the layers in the crystal.

The results of a morphology prediction for the three forms of the free base of Venlafaxine are presented in chapter 5. Three different methods, the Bravais-Friedel-Donnay-Harker method, the attachment-energy method and a Monte Carlo growth simulation are used for that. A comparison of the predicted and the experimentally found morphologies shows that the Monte Carlo simulation gives a semi-quantitative result for form I and II. In case of form III the correct morphology was found, but some of the predicted indices did not correspond with the experimentally found indices.

In chapter 6 the polymorphic behaviour of the free base of Ondansetron is studied. For Ondansetron, an active pharmaceutical ingredient used to treat vomiting and nausea during chemotherapy, two different crystalline forms are known: ODS-1 and ODS-2. Both forms have been grown from solution. Even using different techniques; XRPD, Nuclear Magnetic Resonance (NMR), and DSC turned out to be not sufficient to determine if Ondansetron shows polymorphism or not. Using a combination of a badly resolved crystal structure of a vapour grown crystal, ODS-3, and molecular modelling, it was hypothesized that the crystal structure of ODS-3 best can be interpreted as a locally ordered solid solution of enantiomers. Because the similarities between the XRPD patterns of the three batches it was assumed that also ODS-1 and
ODS-2 are locally ordered solid solutions of enantiomers. Different incorporations of enantiomers on a mesoscopic scale, in the solid state give rise to slightly different forms of Ondansetron. The amount of water present during the crystal growth induces subtle but important local order in the disorder. The results show in this case that although different ODS forms show different physical properties, one cannot speak of polymorphism.
SAMENVATTING

In dit proefschrift worden verschillende aspecten van polymorfie, het verschijnsel waarbij verbindingen in meer dan een kristalvorm kunnen uitkristalliseren, bestudeerd. Omdat de fysische eigenschappen, o.a. smeltwarmte, warmtecapaciteit en oplosbaarheid, van twee polymorfen kunnen verschillen, wordt er door de farmaceutische wereld veel onderzoek gedaan aan het verschijnsel polymorfie. Dit omdat vooral het oplosbaarheidsverschil van twee polymorfen invloed kan hebben op de effectiviteit, de biobeschikbaarheid en de veiligheid van het medicijn. Veel moeite wordt er dan ook gedaan om de meest stabiele polymorf te vinden, hoewel men nooit helemaal zeker is dat deze gevonden is. Een recent, voorbeeld waarbij het verschil in biobeschikbaarheid van twee polymorfen van belang was, is ritonavir. Nadat het medicijn al bijna twee jaar op de markt was ontstond er een thermodynamisch stabielere polymorph met een lagere oplosbaarheid en dus een lagere biobeschikbaarheid.

Met name de thermodynamische aspecten die een rol spelen bij polymorfie van aktieve bestanddelen van medicijnen worden in dit proefschrift bestudeerd.

Na een algemene inleiding over het fenomeen polymorfie, wordt in hoofdstuk 2 de thermodynamische theorie van de oplosbaarheid van stoffen in oplosmiddelen behandeld, met de nadruk op stoffen die polymorfie vertonen. De relatie tussen fasediagrammen en oplosbaarheid komt daarbij aan de orde. Uitgaande van eenvoudige modellen voor de thermodynamica van oplossingen, waarbij wordt aangenomen dat er geen menging optreedt tussen oplosmiddel en opgeloste stof in de vaste fase, wordt de grote variëteit aan mogelijke vormen van oplosscurven, die het temperatuurgedrag van oplosbaarheid beschrijven, verklaard. Pseudopolymorfen, oftewel solvaten, worden ook behandeld. De modellen monden uit in een formule die het mogelijk maakt om de overgangstemperatuur voor een enantiotroop stelsel van polymorfen te schatten. Hiervoor zijn alleen de smelttemperatuur en de smeltwarmte van de twee polymorfen, beide eenvoudig te bepalen met behulp van Differential Scanning Calorimetry (DSC), nodig.

In het derde hoofdstuk wordt het fasegedrag van de vrije base van Venlafaxine, het aktieve bestandeel van een medicijn dat gebruikt wordt voor de behandeling van stress, bestudeerd. Zowel het polymorfie fasediagram als de thermodynamische relatie tussen de drie vormen wordt bestudeerd. Gebruikte methoden zijn DSC, X-ray Powder Diffraction (XRPD), slurrie experimenten en oplosbaarheidsmetingen. De oplosbaarheid van Venlafaxine werd bepaald in verschillende oplosmiddelen. Er werd vooral gekeken naar de afwijkingen van de oplosbaarheid ten opzichte van de ideale oplosbaarheid volgens de vergelijking van van ’t Hoff.
afwijkingen van de oplosbaarheid bleken van dien aard dat het toekennen van de stabiliteitsgebieden van polymorf I en II, op basis van oplosbaarheidsdata, zou leiden tot een verkeerde toekenning van deze gebieden. Dit is het gevolg van de concave vorm van de oplosbaarheidscurven, een vorm die, vanuit theoretisch standpunt, in meer detail is beschreven in hoofdstuk 2. De overgangstemperatuur voor de polymorfen van Venlafaxine vrije base werd berekend met gebruikmaking van de eerder genoemde vergelijking voor het benaderen van de overgangstemperatuur voor twee polymorfen en vergeleken met de waarden die volgen uit de oplosbaarheidsdata. De concave vorm van de oplosbaarheidscurve is het gevolg van een niet te verwaarlozen temperatuurafhankelijkheid van de smeltwarmte.

In hoofdstuk 4 wordt het opmerkelijke gedrag van de vrije base van Venlafaxine met betrekking tot de polymorfie en chirale resolutie beschreven. Met gebruikmaking van verschillende complementaire technieken werden de drie vormen van de vrije base van Venlafaxine gekarakteriseerd. De kristallen van alle drie de vormen zijn opgebouwd uit nagenoeg identieke enantiomeer zuivere lagen, maar verschillen in stapeling; voor vorm I alternerende bi-lagen van R en S enantiomeren, in het geval van vorm II alternerende dubbele bi-lagen van R en S enantiomeren. Voor vorm III, de vorm met het hoogste smeltpunt, is de enantiomere scheiding volledig, wat leidt tot de vorming van een racemisch conglomeraat. Het racemisch conglomeraat kan worden gegroeid uit een oplossing of via een vast-vast fase overgang van de laagst smeltende polymorf, polymorf I, naar vorm III. Opmerkelijk is dat de vorm van het kristal behouden blijft tijdens de fase overgang. Verondersteld wordt dat de tijdens deze fase overgang optredende chirale scheiding plaatsvindt via een lokaal smeltproces, als gevolg van aanwezige kristalfouten. Het lokale smeltproces maakt het mogelijk dat de moleculen over relatief grote afstanden kunnen migreren tussen de lagen in het kristal.

De resultaten van een morfologie voorspelling voor de drie vormen van de vrije base van Venlafaxine worden gepresenteerd in hoofdstuk 5. Drie verschillende voorspellingsmethoden, te weten de Bravais-Friedel-Donnay-Harker methode, de attachment-energie methode en Monte Carlo groeisimulaties worden gebruikt. Een vergelijking van de voorspelde en de experimenteel gevonden morfologieën laat zien dat de Monte Carlo simulaties een semi-kwantitatief resultaat geven voor polymorf I en II. Voor vorm III wordt de juiste morfologie gevonden maar sommige voorspelde indices komen niet overeen met de experimenteel gevonden indices.

In hoofdstuk 6 wordt het polymorfiegedrag van de vrije base van Ondansetron bestudeerd. Van Ondansetron, een farmaceutisch aktief ingrediënt dat gebruikt wordt om overgeven en misselijkheid tijdens chemotherapie te behandelen, zijn twee verschillende kristallijne vormen bekend; ODS-1 en ODS-2. Beide vormen zijn gegroeid uit de oplossing. Zelfs toepassen van
verschillende technieken, XRPD, Nuclear Magnetic Resonance (NMR) en DSC, was niet voldoende om te kunnen bepalen of in er in dit geval sprake is van polymorfie. Met gebruikmaking van de combinatie van een slecht opgeloste kristalstructuur van een uit de damp gegroeid eenkristal, ODS-3, en molecular modeling wordt geconcludeerd dat de kristalstructuur van ODS-3 het beste kan worden geïnterpreteerd als een lokaal geordend mengkristal van enantiomeren. Omdat de overeenkomsten tussen de XRPD patronen van de drie monsters groot zijn, wordt aangenomen dat ook ODS-1 en ODS-2 lokaal geordende mengkristallen van enantiomeren zijn. Verschillende combinaties van enantiomeren, geïnduceerd door de hoeveelheid water aanwezig tijdens de kristalgroei, veroorzaken mogelijk de verschillende vormen die gevonden worden voor de vrije base van Ondansetron. Uit de resultaten van de studie van de vrije base van Ondansetron kan worden geconcludeerd dat kleine, maar duidelijk aanwezige verschillen niet noodzakelijkerwijs betekenen dat er sprake is van polymorfie.
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- Polymorphism and migratory chiral resolution of the free base of Venlafaxine. A remarkable topotactical solid state transition from a racemate to a racemic conglomerate.

- Polymorphism or structural disorder in Ondansetron?

- The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base.
DANKWOORD

Een promotie onderzoek is normaal gesproken een vervolg van een doctoraal opleiding, en volgt meestal direct na het succesvol afsluiten van deze opleiding. In mijn geval is het iets anders verlopen: na ruim 7 jaar gewerkt te hebben bij het Laboratorium voor Grote Schaal en Service Synthese (LGSS) van de Katholieke Universiteit Nijmegen vervolgde ik mijn carrière in 1994 bij Synthon BV, een farmaceutisch bedrijf werkzaam in het generieke segment.

Om de kennis over polymorfie te verhogen werd in het jaar 2000 professor Piet Bennema aangetrokken als adviseur. Om het niveau op een hoger plan te tillen zou het volgens Piet beter zijn als er diepgaander onderzoek betreffende polymorfie zou worden verricht. Hij dacht aan een samenwerking met de afdeling vaste stof chemie van de Radboud Universiteit in de vorm van een promotieonderzoek.

Liever nog: twee promotieplaatsen, een die zich zou bezighouden met de theoretische kant, een computer modeller, en een die zich zou bezighouden met de praktische kant, het kristalliseren en meten aan de verkregen kristallen. In overleg met Bertus Thijs, het toenmalige afdelingshoofd chemische R&D van Synthonen Jacques Lemmens, directeur van Synthon, werd besloten om de samenwerking gestalte te geven. Op dat moment gaf ik Bertus en Jacques te kennen dat ik wel geïnteresseerd was om de praktische promotieplaats in te vullen.

Na een kort beraad werd akkoord gegaan met dit idee. Ik werd promovendus, zij het dat ik in dienst bleef bij Synthon en gedurende 4 jaar bijna uitsluitend aan onderzoek van voor Synthon interessante verbindingen kon werken. Uiteindelijk duurde het o.a. vanwege persoonlijke omstandigheden iets langer.

Na deze korte inleiding wil ik verschillende mensen bedanken die het uiteindelijk mogelijk hebben gemaakt om mijn onderzoek uit te voeren en af te sluiten met dit proefschrift.

Te beginnen met Jacques Lemmens en Bertus Thijs, die me deze unieke kans hebben geboden om te promoveren. Vanaf het begin hadden zij er het volste vertrouwen in dat het onderzoek succesvol zou worden afgesloten. In een adem wil ik Piet Bennema noemen, die de eigenlijke architect is geweest van het samenwerkingsproject en mij binnen Synthon altijd enthousiast en betrokken heeft begeleid.

Mijn directe collega’s van de afdeling chemische R&D, Arjanne, Renè, Judith, Henar, Jie, Jordy, Rens, Rolf (het huidige hoofd van de afdeling), Jan, Gert Jan en Raymond die altijd vol interesse informeerden naar de voortgang van het onderzoek, wil ik bedanken. Zelfs tijdens de lange periode van het schrijven bleven ze geïnteresseerd.
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Synthon’s vision is to develop, produce and sell high-quality alternatives to innovative medicines. Synthon prefers to concentrate on medicines with complex chemical structures and on medicines which are difficult to formulate.

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