Nucleation, Growth, and Solid Phase Transformations During Precipitation Processes

A dissertation submitted to the
Swiss Federal Institute of Technology Zurich
for the degree of
Doctor of Technical Sciences

presented by
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Zurich 2006
Acknowledgements

First and foremost, I would like to thank Prof. Dr. Marco Mazzotti for accepting me as a Ph.D. student and supervising my work in his group. I have learnt a lot thanks to his scientific support, his joy in mathematical problems and the discussions we had over the last four years.

I gratefully thank Prof. Dr. Gilles Févotte and Dr. Arne Zilian for accepting the task of co-examiner, for the fruitful scientific discussions we had on different occasions and for their interest in my work.

The financial support of Novartis Pharma AG (Basel, Switzerland) is gratefully acknowledged. A special thanks to my main collaborators within Novartis, Mr. Martin Müller and Dr. Jörg Brozio, who contributed significantly during scientific discussions to the presented results. Thanks also to Mr. Dierk Wieckhusen, Dr. Ulrich Meier, Dr. Ricardo Schneeberger, and Dr. Danielle Giron for their valuable input during scientific discussions and also to Dr. Jacques Wyss and Mr. Hans Stücheli, for allowing me to carry out a part of my Raman studies at their laboratories within Novartis.

Many thanks to Mr. Christian Rohrbach and Dr. Werner Dörfler, for their advice, help, and support during the realization of the different experimental facilities and my experimental work. Their technical know-how and valuable suggestions helped a lot to transform ideas to reality. Thanks a lot to Dr. Lars Vicum for the CFD computations, proof
reading and continuous advice throughout my work. Many thanks also to Christian Lindenberg for proof reading and indicating any erroneous computation. A big thanks to Mr. Markus Huber for his continuous help with various analytical measurements and the preparation of SEM photomicrographs, to Mr. Sascha Jovanovic and Mr. Bernhard Cadonau from the Informatics Support Group for their help with computer related issues, as well as to Mr. Martin Meuli for his help with anything dealing with electronics.

A big word of thanks to Davide Bonalumi, who carried out most of the experimental and modeling work presented in the chapter on polymorphic transformation. Grazie Davide, spero che l’articolo ti sia piaciuto! Many thanks also to Adrian Spillmann, Ibrahim Uslu, Véronique Gondoin, and Tim Patey, who were working with me during their semester thesis. I enjoyed our collaboration and have learnt much from the discussions we had.

Thanks a lot to my friends all over Europe and my colleagues at the Institute who have made the last four years a great time. A special thanks to Arvind, for the lively and joyful glimpse of India that we could share with you; Grazie a Barbara e Peter per l’amicizia ”senza confini” che continua anche fino in Spagna, und danke an meine besten Freunde Christian und Andi, die mir trotz meiner regelmässigen Ortswechsel immer zur Seite standen.

Meinen Eltern und meiner Schwester möchte ich für all die Jahre liebenader Unterstützung danken.

Grazie Marina, per il tuo amore, il tuo entusiasmo e la tua pazienza - sono felicissimo di portare il tuo anello.
Abstract

Precipitation processes are used in the fine chemical and pharmaceutical industries mainly for the production of micron and submicron sized organic solid products. The knowledge of the fundamental kinetics governing the precipitation process is essential for effective process design. The aim of this thesis is twofold: on the one hand, fast and robust characterization methods for the measurement of nucleation and growth kinetics were developed using current process analytical technologies. The characterization methods were limited to supersaturation levels where particle formation was not influenced by mixing since the experimental work was elaborated in a stirred batch reactor. On the other hand, the thesis addresses the implementation of the measured kinetics correlations within a population balance based process model in order to simulate the influence of main process parameters on the final product thus enabling stringent process optimization.

Two organic compounds were used as model systems in this work, i.e., L-glutamic acid and PDI747. Firstly, the induction time of the metastable α polymorph of L-glutamic acid was measured during pH-shift precipitation as a function of supersaturation using two in situ monitoring tools, namely ATR-FTIR spectroscopy and FBRM. The growth kinetics of the same polymorph were measured by combining in situ measured desupersaturation data of seeded batch experiments with population balance modeling. Combining the induction time data with the independently measured growth kinetics allowed for the calculation of nucleation rates and for the determination of nucleation kinetics parameters. The implementation of a precipitation model based on pop-
ulation balance equations was used together with the measured kinetics correlations to simulate the L-glutamic acid precipitation process. The comparison of experimental particle size distributions and simulation results indicated the significance of agglomeration during the process.

The knowledge of nucleation and growth kinetics of both, the metastable α and the stable β polymorph of L-glutamic acid was demonstrated to be particularly important at higher temperatures, since a solvent mediated polymorphic transformation process can occur during longer batch residence times. The combination of in situ monitoring data of four different process analytical technologies with population balance modeling yielded the parameters for the kinetics equations. The experimental results and process simulations of the liquid and solid phase concentrations were found to be in good agreement. Further process analysis using the process model allowed for a better understanding of the rate determining fundamental mechanisms of the transformation process.

Finally, the proposed methods for the determination of nucleation and growth kinetics were successfully applied to the antisolvent precipitation of PDI747, an organic compound with a molecular weight typical for active pharmaceutical ingredients. Again, the comparison of process simulations and experimental results indicated the importance of secondary mechanisms during the process, i.e. agglomeration and crystal breakage.
Zusammenfassung


Die Partikelbildungskinetiken zweier Modellsubstanzen, L-Glutaminsäure und PDI747, wurden im Rahmen dieser Arbeit untersucht. Zunächst wurden die Induktionszeiten des metastabilen α Polymorphs der L-Glutaminsäure mit Hilfe zweier in situ Analysetechniken, ATR-FTIR Spektroskopie und FBRM, als Funktion der Übersättigung gemessen. Die Kristallwachstumskinetiken dieses Polymorphs wurden mittels geimpfter Batchexperimente durch die Kombination des


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Nomenclature

Roman symbols

\( A \) [\(-\)] absorbance
\( A \) [\(-\)] baseline integrated area in the Raman spectrum
\( A \) [\(\text{# m}^{-3} \text{s}^{-1}\)] pre-exponential factor in the nucleation rate equation
\( A_{B+S} \) [m s\(^{-1}\)] pre-exponential factor in the surface nucleation growth rate equation
\( A_J \) [\(\text{# m}^{-3} \text{s}^{-1}\)] pre-exponential factor in the nucleation rate equation
\( A_p \) [m\(^2\)] particle surface
\( a \) [m\(^{-1}\)] absorption coefficient
\( a \) [\(-\)] activity
\( a^* \) [\(-\)] activity at equilibrium conditions
\( a_{for} \) [m\(^2\) m\(^{-3}\)] volumetric surface of foreign particles
\( b \) [m] effective path length
\( B_{B+S} \) [\(-\)] exponential factor in the Birth and Spread growth rate equation
\begin{itemize}
    
    \begin{align*}
        C & : \text{[-]} \quad \text{exponential factor in the nucleation rate equation} \\
        C_{BCF} & : [\text{m s}^{-1}] \quad \text{factor in the BCF growth rate equation} \\
        C_J & : \text{[-]} \quad \text{exponential factor in the nucleation rate equation} \\
        c & : [\text{mol m}^{-3}] \quad \text{concentration} \\
        c^* & : [\text{mol m}^{-3}] \quad \text{equilibrium concentration} \\
        c^*_i & : [\text{mol m}^{-3}] \quad \text{polymorphic equilibrium concentration} \\
        D & : [\text{m}^2 \text{s}^{-1}] \quad \text{diffusivity} \\
        D_{BCF} & : \text{[-]} \quad \text{factor in the BCF growth rate equation} \\
        F & : \text{[-]} \quad \text{overall shape factor (} = A_p L V_p^{-1} \text{)} \\
        G & : [\text{m s}^{-1}] \quad \text{growth rate} \\
        G_{B+S} & : [\text{m s}^{-1}] \quad \text{growth rate according to the B+S mechanism} \\
        G_{BCF} & : [\text{m s}^{-1}] \quad \text{growth rate according to the BCF mechanism} \\
        I_0 & : \text{[-]} \quad \text{emitted radiation} \\
        I & : \text{[-]} \quad \text{transmitted radiation} \\
        J & : [\text{# m}^{-3} \text{ s}^{-1}] \quad \text{nucleation rate} \\
        K & : \text{[-]} \quad \text{equilibrium constant} \\
        K_n & : \text{[-]} \quad \text{exponential factor in the nucleation rate} \\
        K_g & : \text{[-]} \quad \text{exponential factor in the growth rate} \\
        K_{s\beta} & : \text{[-]} \quad \text{exponential factor in the } \beta \text{ surface nucleation rate} \\
        k & : [\text{J K}^{-1}] \quad \text{Boltzmann constant} \\
        k_a & : \text{[-]} \quad \text{surface shape factor} \\
        k_d & : [\text{m s}^{-1}] \quad \text{mass transfer coefficient} \\
        k_g & : [\text{m s}^{-1}] \quad \text{pre-exponential factor in the growth rate}
    \end{align*}
\\
\end{itemize}
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_i$</td>
<td>$[# \ s^{-1}]$</td>
<td>impingement rate</td>
</tr>
<tr>
<td>$k_{if}$</td>
<td>$[# \ s^{-1}]$</td>
<td>impingement rate based on foreign particle surface</td>
</tr>
<tr>
<td>$k_n$</td>
<td>$[# \ m^{-3} \ s^{-1}]$</td>
<td>pre-exponential factor in the nucleation rate</td>
</tr>
<tr>
<td>$k_{s\beta}$</td>
<td>$[# \ m^{-2} \ s^{-1}]$</td>
<td>pre-exponential factor in the $\beta$ surface nucleation rate</td>
</tr>
<tr>
<td>$k_v$</td>
<td>[-]</td>
<td>volume shape factor</td>
</tr>
<tr>
<td>$L$</td>
<td>[m]</td>
<td>crystal size</td>
</tr>
<tr>
<td>$L'$</td>
<td>[m]</td>
<td>crystal size of bin $n+1$ in the PBEs</td>
</tr>
<tr>
<td>$M$</td>
<td>[kg mol$^{-1}$]</td>
<td>molar mass of solute</td>
</tr>
<tr>
<td>$m_2^\alpha$</td>
<td>$[m^2 \ m^{-3}]$</td>
<td>second moment of the $\alpha$ particle size distribution</td>
</tr>
<tr>
<td>$N_e$</td>
<td>[-]</td>
<td>number of experiments</td>
</tr>
<tr>
<td>$N_p$</td>
<td>[-]</td>
<td>number of experimental values</td>
</tr>
<tr>
<td>$n(L,t)$</td>
<td>$[# \ m^{-4}]$</td>
<td>number density in the PBEs</td>
</tr>
<tr>
<td>$n_c$</td>
<td>$[# \ m^{-4}]$</td>
<td>critical nuclei number density</td>
</tr>
<tr>
<td>$n_{cf}$</td>
<td>$[# \ m^{-3}]$</td>
<td>critical nuclei number density based on foreign particle surface</td>
</tr>
<tr>
<td>$q$</td>
<td>[-]</td>
<td>condensation coefficient</td>
</tr>
<tr>
<td>$R$</td>
<td>[J mol$^{-1}$ K$^{-1}$]</td>
<td>ideal gas constant</td>
</tr>
<tr>
<td>$S$</td>
<td>[-]</td>
<td>relative supersaturation ($= c/c^*$)</td>
</tr>
<tr>
<td>$S_{exp}$</td>
<td>[-]</td>
<td>experimental supersaturation values</td>
</tr>
<tr>
<td>$S_{mod}$</td>
<td>[-]</td>
<td>calculated supersaturation values</td>
</tr>
<tr>
<td>$Sc$</td>
<td>[-]</td>
<td>Schmidt number</td>
</tr>
<tr>
<td>$T$</td>
<td>[K]</td>
<td>absolute temperature</td>
</tr>
<tr>
<td>$t$</td>
<td>[s]</td>
<td>time</td>
</tr>
</tbody>
</table>
\[ t_i \quad [s] \quad \text{induction time} \]
\[ V_m \quad [m^3 \text{ mol}^{-1}] \quad \text{molecular volume} \]
\[ V_p \quad [m^3] \quad \text{particle volume} \]
\[ W^* \quad [J] \quad \text{nucleation work} \]
\[ w \quad [m \text{ s}^{-1}] \quad \text{velocity} \]
\[ x_\alpha \quad [-] \quad \text{mass fraction of suspended } \alpha \text{ crystals} \]
\[ Z \quad [-] \quad \text{Zeldovc imbalance factor} \]

**Greek symbols**

\[ \alpha_v \quad [-] \quad \text{detectable volume fraction} \]
\[ \Delta C \quad [\text{kg} \text{ m}^{-3}] \quad \text{concentration difference } (= C - C^*) \]
\[ \Delta G \quad [\text{J} \text{ mol}^{-1}] \quad \text{Gibbs free energy} \]
\[ \Delta \mu \quad [\text{J} \text{ mol}^{-1}] \quad \text{chemical potential} \]
\[ \bar{\epsilon} \quad [\text{W} \text{ kg}^{-1}] \quad \text{average power input} \]
\[ \gamma \quad [\text{J} \text{ m}^2] \quad \text{interfacial tension} \]
\[ \nu \quad [\text{m}^2 \text{ s}^{-1}] \quad \text{kinematic viscosity} \]
\[ \rho_c \quad [\text{kg/m}] \quad \text{crystal density} \]
Superscripts and subscripts

A surface

b amine group of the L-glutamic acid molecule

for based on foreign particle surface

Het Heterogeneous

Hom Homogeneous

V volume

w water molecule

α α polymorph

α α carboxyl group of the L-glutamic acid molecule

β β polymorph

γ γ carboxyl group of the L-glutamic acid molecule

Abbreviations

ATR-FTIR Attenuated total reflection Fourier transform infrared

CLD Chord length distribution

FBRM Focussed Beam Reflection Measurement

PSD Particle size distribution

SEM Scanning electron microscopy
Chapter 1

Introduction

Precipitation is the rapid crystallization of sparingly soluble materials, usually as a result of a chemical reaction or of physical changes in a solution. Although precipitation processes are widely used in the chemical, fine chemical and pharmaceutical industry, thorough process design is rarely possible due to the unknown kinetics of the fundamental phenomena involved. Moreover, the particle formation mechanisms during precipitation processes can become influenced by the mixing of the participating fluids at high supersaturation levels. Yet, these supersaturation levels vary for every system and reactor geometry and the risk of mixing influences increases with rising supersaturation. Consequently, every precipitation process can be performed at lower supersaturation levels, where no mixing influence occurs for a given reactor geometry. Thorough process design necessitates the kinetics of particle formation and growth in any case, whereas the description of the mixing process in the reactor from the micro-, and meso-, to the macroscale is needed only for mixing influenced precipitation processes [1]. In this work the supersaturation levels of each system were limited to a maximum value since the particle formation kinetics were characterized in common stirred batch reactors and any mixing influence had therefore to be excluded. This introductory chapter is divided into five sections: section 1.1 is devoted to the main fundamental mechanisms that occur during precipitation
processes, i.e., nucleation and growth; sections 1.2 and 1.3 highlight the importance and give an overview of the literature published in the field of polymorphism and process analytical technologies (PATs), respectively; section 1.4 shortly describes the two model substances used in this work; whereas section 1.5 gives the objectives and the outline of this thesis.

1.1 Fundamental mechanisms during precipitation processes

Nucleation and growth are key mechanisms that determine the final crystal size, crystal size distribution and crystal shape of particulate products. Determining the rate of these processes is an important step in crystallization and precipitation process design and development. In particular the characterization of nucleation rates has proven to be challenging and up to now no standard measurement procedure could be established. The following sections contain the theoretical approaches to describe particle nucleation and growth and indicate some important publications on both topics.

1.1.1 Nucleation

Nucleation is the initial process during new phase formations such as condensation, crystallization, and precipitation. First publications on nucleation kinetics date back to 1926 [2]. The classical nucleation theory describes nucleation by the collision of molecules due to Brownian motion. These collisions lead to the formation of clusters in a supersaturated environment. This dynamic process and the attachment and detachment of further molecules depends on the supersaturation level. The free enthalpy of the formed clusters can be calculated as the sum of the free enthalpies of the newly created surface $\Delta G_A$ and the cluster volume $\Delta G_V$:

$$\Delta G = \Delta G_A + \Delta G_V. \quad (1.1)$$

If it is assumed that the formed cluster is of spherical shape, $\Delta G_A$, the enthalpy needed to create the new phase boundary, can be defined as
the product of the spherical cluster surface and the interfacial tension $\gamma$:

$$\Delta G_A = 4\pi r^2 \gamma \quad (1.2)$$

where $r$ is the cluster radius. The volume free enthalpy $\Delta G_V$ can be accordingly defined as the product of the cluster volume and the difference in the chemical potential $\Delta \mu$ between the liquid and the solid phase divided by the molecular volume $V_m$:

$$\Delta G_V = -\frac{4\pi r^3}{3V_m} \Delta \mu \quad (1.3)$$

Combining Eqs. 1.1-1.3 results in:

$$\Delta G = 4\pi r^2 \gamma - \frac{4\pi r^3}{3V_m} \Delta \mu \quad (1.4)$$

The difference in chemical potential between liquid and solid phase is generally defined as

$$\Delta \mu = RT \ln S \quad (1.5)$$

where $R$ denotes the ideal gas constant, $T$ the absolute temperature and $S$ the activity based supersaturation

$$S = \frac{a}{a^*} \quad (1.6)$$

with $a$ being the actual activity and $a^*$ the equilibrium activity. A thermodynamically stable cluster exists when the free enthalpy $\Delta G$ does not change when a single molecule is added or removed, i.e.,

$$\frac{\partial \Delta G}{\partial r} = 0. \quad (1.7)$$

Differentiation of Eq. 1.4 allows for the calculation of the critical cluster radius $r^*$

$$r^* = \frac{2\gamma V_m}{RT \ln S} \quad (1.8)$$

Substituting Eq. 1.8 in Eq. 1.4 allows for the calculation of the work necessary to form a nucleus with the size of the critical cluster $W^*$

$$W^* = \Delta G(r^*) = \frac{16\pi \gamma^3 V_m^2}{3(RT \ln S)^2} \quad (1.9)$$
According to the classical nucleation theory the rate of primary homogeneous nucleation can be calculated with the number of critical clusters by

\[ J_{Hom} = n_c k_i Z \]  \hspace{1cm} (1.10)

where \( n_c \) denotes the number concentration of clusters with the radius \( r^* \), \( k_i \) is the impingement rate of molecules on the cluster surface and \( Z \) is the Zeldovic factor, which accounts for the equilibrium distortion. Assuming that the number concentration of clusters with critical radius \( n_c \) is caused by random collisions of molecules it can be described using a Boltzmann distribution

\[ n_c = n_0 \exp \left( -\frac{\Delta G_c}{kT} \right) \]  \hspace{1cm} (1.11)

with \( n_0 \) being the number concentration of molecules in the supersaturated solution and \( k \) being the Boltzmann constant. The impingement rate \( k_i \) can be described as:

\[ k_i = A n q \frac{w}{A} \]  \hspace{1cm} (1.12)

where \( A \) is the surface area of the nucleus, \( n \) is the number concentration of molecules, \( q \) is a condensation coefficient, and \( w \) denotes the average velocity of the molecules. Combining Eqs. 1.10-1.12 yields the functional relationship between the primary homogeneous nucleation rate \( J_{Hom} \) and the supersaturation \( S \)

\[ J_{Hom} = A_{Hom} \exp \left( -\frac{C_{Hom}}{\ln^2 S} \right). \]  \hspace{1cm} (1.13)

A second type of primary nucleation is the heterogeneous nucleation on foreign particles, which was described for gas [3] and solid phase nucleation mechanisms [4, 5]. In general, heterogeneous nucleation occurs on the surface of foreign solid matter which is in contact with the solution. It was shown, that even in double distilled water many SiO\(_2\) nanoparticles exist. The heterogeneous nucleation rate \( J_{Het} \) is the product of the
1.1 Fundamental mechanisms during precipitation processes

volumetric surface $a_{for}$ of foreign particles and the rate of heterogeneous nucleation based on the surface of foreign particle $J_{for}$:

$$J_{Het} = a_{for} J_{for}$$  \hspace{1cm} (1.14)

where $J_{for}$ can be described in equivalence to the homogeneous nucleation rate in Eq. 1.10:

$$J_{for} = n_{cf} k_{if} Z_{for}.$$  \hspace{1cm} (1.15)

The factor $n_{cf}$ is the number of critical nuclei based on the surface of foreign particles, whereas $k_{if}$ and $Z_{f}$ denote the corresponding impingement rate and imbalance factor. Thus, the functional form of the heterogeneous nucleation rate is the same as for homogeneous nucleation given in Eq. 1.13 and is defined as:

$$J_{Het} = A_{Het} \exp \left( \frac{-C_{Het}}{\ln^2 S} \right).$$  \hspace{1cm} (1.16)

The functional forms given in Eqs. 1.13 and 1.16 will be used to describe primary homogeneous and heterogeneous nucleation in this work and the objective is to determine the corresponding kinetic parameters, i.e., $A_{Hom}$, $C_{Hom}$, $A_{Het}$, and $C_{Het}$.

1.1.2 Crystal growth

Crystal growth is the mechanism responsible for the reduction of the initial supersaturation, whereas nucleation determines the number of particles in the system. In case of high nucleation rates and low growth rates, a large number of small particles will be produced, whereas the opposite case will lead to the formation of fewer and larger crystals. The growth of crystals in a supersaturated solution is a complex process that can be summarized with consecutive diffusion and integration steps following the approach of diffusion and reaction used in chemical reaction engineering. Consequently, crystal growth can be either diffusion limited or integration controlled or a mixture of both mechanisms. Generally, the
linear growth rate $G$ is defined as the change of a characteristic crystal dimension per unit time:

$$G = \frac{dL}{dt} \quad (1.17)$$

where $L$ denotes the characteristic length of the crystal and $t$ is the time. Assuming that the integration reaction of new growth units in the crystal lattice as infinitely fast, crystal growth is limited by the diffusive-convective transport and the diffusion limited growth rate $G_{\text{dif}}$ can be defined as:

$$G_{\text{dif}} = k_d \frac{k_a}{3k_v \rho_c} \Delta c \quad (1.18)$$

where $k_d$ is the mass transfer coefficient, $\rho_c$ denotes the crystal density, $\Delta c = c - c^*$ is the concentration based supersaturation, and $k_a$ and $k_v$ denote the surface and volume shape factors, respectively [6]. It is worth noting that the growth rate given in Eq. 1.18 represents the maximum growth rate, since every growth unit has to be transported to the crystal surface before it can be integrated in the crystal lattice.

In case of integration controlled crystal growth, the integration of new growth units in the crystal lattice is slower than the transport of new growth units to the crystal surface. Two different integration mechanisms are often used in literature to describe integration controlled growth: a screw dislocation mechanism where the integration of new growth units takes place at energetically favorable integration sites and a surface nucleation mechanism, which result from the collision of several growth units which eventually form a stable nuclei on the crystal surface. For the screw dislocation mechanism a step model was proposed by Burton Cabrera and Frank (BCF) which is defined as [7]:

$$G_{\text{BCF}} = C_{\text{BCF}} \left( (S - 1)^2 / D_{\text{BCF}} \right) \tanh(D_{\text{BCF}} / (S - 1)) \quad (1.19)$$

where $C_{\text{BCF}}$ and $D_{\text{BCF}}$ are system dependent parameters. A popular model for the surface nucleation growth mechanism is the so called birth and spread (B+S) model which is defined as:

$$G_{\text{B+S}} = A_{\text{B+S}} (S - 1)^{5/6} \exp(-B_{\text{B+S}} / (S - 1)) \quad (1.20)$$

where $A_{\text{B+S}}$ and $B_{\text{B+S}}$ are factors that have to be determined experimentally for a given system. Besides the B+S model further surface nucleation growth models such as the mononuclear and polynuclear models
have been proposed. In this work, the B+S model is the only surface nucleation model considered, since it is an intermediate approach between the two borderline cases described by the mono- and polynuclear models.

1.1.3 Measurement of nucleation and growth kinetics

The accurate determination of nucleation and growth kinetics for precipitation processes is not a straight forward task, since particularly the nucleation step can be influenced by mixing mechanisms from the micro-, to the meso- and macro-scale depending on the level of supersaturation. A good overview about the various methods proposed to determine nucleation and growth kinetics of crystallization processes was published by the Working Party on Crystallization of the European Federation of Chemical Engineering [8].

The measurement of fast nucleation rates was performed mainly with a couple of inorganic systems like BaSO$_4$ and CaCO$_3$ which were used as model compounds over decades during the last century [9, 10, 11, 12]. Due to the difficulties occurring during the determination of fast nucleation rates only a limited number of organic systems was studied, i.e., benzoic acid [13, 14], salicylic acid [15], H$_2$EDTA [16], L-asparagine monohydrate and Lovostatin [17, 18]. Summarizing, different approaches have been proposed for the measurement of nucleation kinetics for example methods using combined particle counting and process time measurements [19, 20], MSMPR experiments in combination with particle size distribution measurements [8] or simultaneous nucleation and growth rate characterization from batch experiments using population balance modeling [15, 21, 22]. Recently, the different approaches to determine nucleation kinetics of fast precipitation processes were compared to each other [23].

Once nucleation and growth kinetics are determined for a given system, the kinetics correlations can be combined with population balance modeling and computational fluid dynamics (CFD) for the simulation and scale-up of the process [24, 25, 26].
1.2 Polymorphism and solid phase transformation

Polymorphism is the ability of a solid material to exist in more than one crystal structure. A prominent example of a solid material exhibiting various crystal structures is carbon, which can exist as graphite, diamond, carbon nanotubes or fullerenes. Yet, in the case of elements multiple solid structures are called "allotropy", whereas the term "polymorphism" applies to all other compounds including molecules, polymers, and metals. Two kinds of polymorphism are distinguished: "packing polymorphism" exists when the molecule is arranged in different crystal structures, whereas "conformational polymorphism" describes the existence of different conformers of the same molecule in different polymorphic modifications which are generally characterized by a low energy difference between the various conformations. Glycine is an example of packing polymorphism, where the \( \alpha \) polymorph is arranged in a monoclinic crystal lattice while the \( \gamma \) form has a hexagonal lattice [27]. Conformational polymorphism is featured for example by L-glutamic acid [28] or the drug substance Spiperone [29]. In any case only one polymorphic form is the thermodynamically stable form at a given temperature and pressure. The difference in stability of different polymorphs results in different physical and chemical properties, such as melting point, solubility, and density, which in turn can have significant effects on various product properties such as flowability, bioavailability, compressibility and dissolution rate. Consequently, polymorphism is of relevance in industries where such product properties are of highest importance including pharmaceutical products, agrochemicals, pigments, foods, and explosives [30, 31, 32]. When inquiring about the number of possible polymorphs of a substance one should refer to what is known as McCrone’s law, which states that "Every compound has different polymorphic forms, and that, in general, the number of forms known for a given compound is proportional to the time and money spent in research on that compound." [33].

Despite the importance of polymorphs, the systematic characterization of nucleation and growth kinetics of different polymorphs is still in the fledgling stages. The main comment concerning polymorph crystalliza-
tion kinetics repeatedly found in literature is that a system behaves "according" [34, 35] or "in contradiction to Ostwald’s rule of stages" [36, 37]. Wilhelm Ostwald, a German-baltic chemist born 1853 in Riga and honored 1909 with the Nobel prize in Chemistry for his work about catalysis, published in 1897 an article with the title: "Studien über die Bildung und Umwandlung fester Körper" (Studies of the formation and transformation of solid substances) [38]. In this article, Ostwald proposed that the solid formed first during a crystallization process would be the least stable polymorph, which subsequently transforms into the more stable polymorphs over time. Recently, the Ostwald’s rule of stages was illustrated on the basis of a structural analysis and irreversible thermodynamics [39]. Yet, it is worth noting that "Ostwald’s rule" is not a general law and the exceptions can be understood within the theoretical framework discussed of irreversible thermodynamics [39]. However, the classification of a polymorphic system with respect to Ostwald’s rule of stages is not sufficient for rigorous process design. Another approach to characterize the crystallization behavior of a polymorphic system is to analyze the polymorphic composition as a function of time under different process conditions [40, 41, 42, 43, 44, 45, 46, 47]. Only few papers have been devoted to the determination of nucleation [48, 49, 50] and growth kinetics [51, 52, 53] of organic polymorphic systems and even less articles were published, where nucleation and growth kinetics were combined with population balance modeling to simulate the crystallization and transformation process and to validate the determined kinetics for inorganic [54, 55] and organic systems [50, 56]. Yet, the case of the pharmaceutical drug Ritonavir, where the appearance of an unknown polymorphic form two years after the market launch of the product had drastic economic consequences, is only one example that indicates the need for careful and rigorous kinetics characterization of polymorphic systems [57].

1.3 Process analytical technologies

Process analytical technologies have been used in the petrochemical industry already for several decades and have recently become important also in the fine chemical and pharmaceutical industries for the anal-
ysis, optimization, and control of day-to-day performance of chemical processes. An in situ process analytical technology (PAT) is commonly defined as a "... system for designing, analyzing and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" [58]. The reason why PATs are so attractive for process analysis and control is obvious: in industries with very high production rates (petrochemical) or very high added values (pharmaceutical) each minute of erroneous production reduced by online measurements compared to offline analysis translates directly into lower production costs [59, 60]. A series of review articles covers the development of a broad array of different process analytical technologies in the decade from 1993 to 2003 [61, 62, 63, 64, 65]. Moreover, the US Food and Drug Administration (FDA), which is the regulatory organization responsible for the approval of food and pharmaceutical products in the US, has recently launched the so called PAT initiative to promote the use of process analytical technologies during the manufacturing processes in the pharmaceutical industries [58]. According to the FDA the goal of PAT is "... to understand and control the manufacturing process, which is consistent with our current drug quality system: quality cannot be tested into products; it should be built-in or should be by design." In this work, different PATs are used to monitor liquid and solid phase properties such as concentration, particle size, and polymorphic form to prevent offline analysis which could introduce substantial errors by sampling. The different PATs used are described in detail in Section 2.2.

1.4 Model compounds

Two different model compounds were used in this work, i.e., L-glutamic acid and PDI 747. L-glutamic acid, C₅H₉NO₄, is a small amino acid that plays an important role in the human biology. The substance features two polymorphs, the metastable α polymorph and the stable β form; the crystal structures are both orthorhombic, \(P2_12_12_1\), with the crystal lattice parameters be-
ing \( a = 0.706, b = 1.03, c = 0.875 \) nm and \( a = 0.517, b = 1.734, c = 0.695 \) nm, respectively [66, 67]. L-glutamic acid was chosen since there were already several articles about the polymorphic transformation published which were taken as reference for this work. Yet, no nucleation and overall growth kinetics were known when this thesis was started. PDI 747 was proposed as a typical API candidate by Novartis, the industrial partner of this project.

1.5 Objectives and outline of the thesis

This thesis is the result of a joint project between the Separation Processes Laboratory of Prof. Dr. Marco Mazzotti at the Federal Institute of Technology Zurich and Novartis Pharma AG. The ultimate objective of this work is to develop robust characterization methods for nucleation, growth, and transformation phenomena that take place during precipitation processes. Once the kinetics are determined for a specific system, the kinetics of the participating fundamental mechanisms are combined with population balance modeling to allow for process simulation, analysis, and synthesis. Finally, the comparison of experimental and simulation results is used to validate the determined kinetics parameters.

The experimental set-up designed and constructed in the framework of this thesis is described in Chapter 2. This chapter includes as well detailed descriptions of the analytical techniques and devices that were employed to measure different liquid and solid phase properties of the different model compounds.

Chapter 3 addresses the issue of accurate and robust determination of nucleation kinetics. The proposed method is based on induction time measurements and adopts two different in situ process analytical techniques, namely ATR-FTIR and FBRM. The method is applied to the pH-shift precipitation of L-glutamic acid upon mixing of an aqueous monosodium glutamate solution with hydrochloric acid.

Chapter 4 describes the independent measurement of growth kinetics. The method employs seeded batch desupersaturation experiments combining again ATR-FTIR and FBRM, and is applied to the growth of
metastable $\alpha$ L-glutamic acid crystals. Different experimental parameters are varied to validate the absence of size dependent growth. The experimental data is combined with a population balance model and an optimization routine to determine the growth rate parameters.

Chapter 5 introduces a process model for precipitation processes. The nucleation and growth kinetics during the pH-shift precipitation of L-glutamic acid determined in Chapters 3 and 4 are used to simulate the precipitation process as a function of initial supersaturation. The comparison of model and experimental results indicates the importance of secondary mechanisms, i.e. agglomeration.

Chapter 6 is dedicated to the polymorphic transformation of L-glutamic acid. It is worth noting that major parts of the experimental and modeling work were carried out by the co-author of the published article, Davide Bonalumi. Four different in situ process analytical techniques, ATR-FTIR, FBRM, in situ microscopy and Raman spectroscopy are used to measure liquid and solid phase data during seeded and unseeded transformation experiments at different initial supersaturation values. The experimental data is combined with a population balance model to estimate the kinetics of the different mechanisms involved. Model simulations and experimental observations reveal the rate limiting step to be the nucleation of the stable $\beta$ polymorph on the surface of the metastable $\alpha$ form.

Chapter 7 describes the application of nucleation and growth kinetics characterization methods introduced in Chapters 3 and 4 to the anti-solvent precipitation of PDI 747. A precipitation model based on the population balance equation is used to simulate the process at different initial supersaturation values. The validity of the measured kinetics is assessed by comparison of simulation and experimental results.

Chapter 8 discusses the conclusions of this work and provides an outlook for the future research in this area.
Chapter 2

Experimental set-up and analytical techniques

This chapter describes the experimental set-up used throughout this work and illustrates the basic principles and advantages of the key in situ and off line analytical techniques. The chapter is organized as follows: section 2.1 describes the different reactors developed for the characterization and precipitation experiments. The various in situ analytical technologies employed are highlighted in section 2.2 whereas the off line analytical devices are presented in section 2.3.

2.1 Experimental set-up

2.1.1 Stirred batch reactor

All experiments described in this work were performed in stirred batch glass reactors of 0.25, 0.5, or 2 L volume. Since stainless steel is known to enhance secondary nucleation in comparison to teflon or glass [68, 69, 70], all reactors and stirrers used in this work were made of pyrex glass in order to improve experiment to experiment reproducibility. The double
wall reactors were purchased from LTS (Basel, Switzerland) while GPE Limited (Bedfordshire, UK) delivered high precision pyrex glass stirrers. The dimensions of reactors and stirrers are given together with the maximum number of probes for each reactor in Table 2.1. The temperature in the 0.25 and 0.5L reactors was controlled using a CC230 thermostat (Huber, Offenburg, Germany) and the 2L reactor was temperature controlled using a CC240 WL-3 thermostat (Huber, Offenburg, Germany), all connected via a Pt 100. The reactors are designed in such a way, that the reactor lid is mounted in a fixed position, while the reactor itself is connected to a vertical rail and can easily slide up and down which facilitates experimental operation and cleaning particularly with respect to the implementation of various in situ probes in the reactor.

<table>
<thead>
<tr>
<th>Reactor volume</th>
<th>Reactor ID</th>
<th>Stirrer diameter</th>
<th>Max. number of probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 L</td>
<td>63 mm</td>
<td>30 mm</td>
<td>1</td>
</tr>
<tr>
<td>0.5 L</td>
<td>100 mm</td>
<td>50 mm</td>
<td>3</td>
</tr>
<tr>
<td>2 L</td>
<td>145 mm</td>
<td>70 mm</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.1: Dimensions of the different stirred batch reactors.

2.1.2 Y-mixer set-up

Besides the stirred batch reactors presented in the previous section, a continuous precipitation reactor with a small confined mixing chamber was designed, that allows for the realization of micro mixing times in the order of milliseconds, thus excluding mixing effects during precipitation at high levels of the supersaturation. None of the results presented in this work were obtained using this device, however, it is planned to determine nucleation rates at high levels of supersaturation and to compare them to the nucleation rates determined by induction time measurements at lower levels of $S$. The main objective of the continuous Y-mixer set-up was to assure very small micro-mixing times in the range of 1 to 5 ms, in order to prevent any mixing effects on the precipitation. Besides,
any backmixing in the reaction tube had to be avoided. Moreover, no static mixers were planned in a first approach because of an increased clogging risk in the set-up. Finally, to allow for defined supersaturation conditions in the mixing chamber, the liquids had to be pumped into the mixing chamber at high pressure and minimal flow fluctuations. Except rather costly high pressure syringe pumps no commercial pumping solution was available that fulfilled these requirements. Therefore we decided to use two Pneumatex PWD 8 pressure tanks with a maximum pressure of 25 bar and a maximum liquid storage volume of 6.4 liters to provide the necessary stable liquid pressure. Fig. 2.1 shows the flow sheet of the experimental set-up. Before each experiment, the liquids are pumped through the valves V-4 and V-5 and the heat exchangers 1 and 2 into the depressurized tanks. The liquids pass through these heat exchangers during the filling process as well as during the experiment. Both tanks as well as the tubing between the tanks and the Y-mixer are
thermally insulated, to guarantee that the liquids enter the Y-mixer at the desired temperature. To start an experiment, the pressure tanks are pre-pressurized with a conventional nitrogen gas bottle. The gas pressure is set with a VUH pressure reducing regulator from Lüdi (Regensdorf, Switzerland). As this regulator delivers a fixed pressure, no control loop was used to prevent possible pressure fluctuations. However, the pressure on the gas side is measured throughout the whole experiment. Between the pressure tanks and the mixer the liquid flow rate, the pressure, and the liquid temperature are measured in order to verify that both liquids reach the mixer at the chosen conditions. The nucleation kinetics can be determined using the Y-mixer set-up in combination with a suitable quenching tank and a particle counting technique such as the Coulter counter technique presented in section 2.3.1. Fig. 2.2 shows the scheme of the set-up combination. The Y-mixer is used to mix the reactants sufficiently fast in order to prevent the influence of mixing conditions on the precipitation kinetics. The residence time of the product stream in the precipitation tube is kept short to maintain a constant level of supersaturation. Finally, the quenching tank is used to stop the particle formation. It is worth noting, that the quenching time $t_{\text{quench}}$ has to be much smaller than the residence time $t_r$. The time scale of quenching can

Figure 2.2: Experimental set-up to determine nucleation kinetics
be estimated using an established CFD model of the quenching tank. Before the precipitated nuclei can be counted, they have to be grown to detectable size preventing all mechanisms that change the particle number, i.e., further nucleation, agglomeration, breakage, and attrition. After the growth step, a representative sample is analyzed off-line by particle counting for different residence times. Finally, the number of particles $N_p$ yields the nucleation rate $B$ through the following relation:

$$ B = \frac{N_p}{(Q \cdot t_{exp}) \cdot t_r} \quad (2.1) $$

where $Q$ denotes the volume flow rate of the product stream, $t_{exp}$ the duration of the experiment, and $t_r$ the residence time of the product stream in the precipitation tube.

### 2.1.3 Y-mixer CFD study

The main goal of the experimental Y-mixer set-up was to realize mixing conditions that allow for the fast mixing of two reactant streams on all relevant mixing scales. In order to characterize the mixing quality, two different mixing geometries, a simple Y-mixer and a Roughton type mixer, were analyzed using computational fluid dynamics (CFD). The finite volume based CFD software FLUENT 6.1 was employed to solve the model equations by discretizing the Y-mixer and the Roughton mixer into 154,411 and 161,335 cells, respectively. The flow field was calculated using the Reynolds-stress model since the flow field is strongly non-homogeneous in the mixer.

#### Y-mixer geometry

The Y-mixer geometry was designed after analyzing mixer designs published in other precipitation studies [15, 20, 71]. We designed our Y-mixer with an inlet tube diameter of 0.5 mm and an impingement angle $\alpha = 160^\circ$, while the outlet tube has an inner diameter of 1 mm and is 15 mm long as shown in Fig. 2.3 a). Fig. 2.3 b) shows the mixer geometry as implemented in the CFD code. Fig. 2.4 shows the results of the
2. Experimental

Figure 2.3: a) Y-mixer geometry; b) implemented in FLUENT

CFD analysis for equal reactant mass flows of 5 g/s, namely a) the mixture fraction distribution and b) the spatial residence time distribution. The mixture fraction distribution is a color-coded representation of the mixing quality being normalized to the inlet stream A. Thus, the red color depicts pure stream A while the blue color depicts the absence of A, thereby pure stream B. Maximum energy dissipation occurs at the point where both streams impinge, resulting in instantaneous small scale mixing at the contact plane between both liquids. Yet, the macromixing in this region is rather unsatisfactory since both streams are highly segregated and large volumes of each stream are not mixed whilst entering the precipitation tube. A more uniform mixing pattern develops while the liquid flows down the outlet tube. On the right side of the picture the corresponding residence times are shown. From the comparison of both images it can be concluded, that both streams have almost reached complete mixing after 1.5 ms for this mass flow conditions. For anti-solvent precipitation processes there might be the need to mix solution and anti-solvent at a mass ratio different from 1:1. Therefore, we performed a similar analysis for a mass flow ratio of 3:1. Fig. 2.5 a) and b) show the mixture fraction distribution and the corresponding residence time for this case, respectively. While the residence time is very similar to the results at equal mass flow, the mixture fraction distribution reaches a more uniform level than in the former case. This can be explained by the vicinity of the impinging zone to the tube wall. Since the shear forces close to the wall are higher than in the tube center, the backmixing with
2.1 Experimental set-up

Figure 2.4: a) Dimensionless mixture fraction distribution and b) residence time in the Y-mixer calculated for equal mass flows of 5 g/s for each inlet flow.

The bulk liquid is more intense and results in a faster mixing of both liquids compared to the case with equal mass streams. Therefore, complete mixing is achieved already in less than one millisecond.

Optimized Roughton type geometry

Söhnel and Garside proposed different rapid mixing devices, e.g. a Roughton mixing chamber for precipitation studies with fast nucleation and growth kinetics [72]. In order to evaluate the mixing performance of such a mixer, we analyzed an optimized Roughton geometry through CFD. Fig. 2.6 shows the geometry of the Roughton mixer implemented in the CFD grid. Fig. 2.7 shows the results of the study for equal inlet flows of 5 g/s. As it can be seen from the mixture fraction distribution,
both inlet streams are mixed completely within the mixing chamber. No significant concentration differences can be observed once the mixture has entered the precipitation tube. The right-hand side of the graph displays the associated residence times from which it can be concluded, that complete mixing can be achieved faster than 0.5 ms in the Roughton mixer at the given flow rates. Comparing both mixer geometries it can be concluded, that the Roughton chamber is able to perform faster and more efficient mixing than the Y-mixer. This is mainly due to its better mixing abilities on the meso- and macro-mixing scale. Although the residence time distribution in the Roughton type mixer is slightly broader than in the Y-mixer case, this should not have any significant impact during precipitation experiments.
Figure 2.6: Optimized Roughton mixer geometry implemented in a CFD grid

Figure 2.7: a) Dimensionless mixture fraction distribution and b) residence time in seconds calculated for the optimized Roughton geometry using equal mass flows of 5 g/s for each inlet flow.
2.2 In situ process analytical technologies

Four different in situ PAT have been used in this work: Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy, Focused Beam Reflectance Measurement (FBRM), in situ microscopy and Raman spectroscopy. Each of these techniques is presented subsequently in the following subsections.

2.2.1 ATR-FTIR spectroscopy

Infrared spectroscopy is based on the phenomenon that molecules interact with light in the range between 1 and 1000 μm. Mid-IR spectroscopy uses light with a wavelength of about 5 to 20 μm where a part of the IR light is absorbed by vibrations and rotations of the molecules at different frequencies depending on the molecular structure of the absorbing substance. In principle, the vibrational frequencies of a molecule can be entirely calculated taking into account the masses of all vibrational centers, the power constants of all chemical bonds and the geometry of the molecule. Yet, such calculations require huge computational efforts for larger molecules. Nowadays, one main application area of IR spectroscopy is the molecular and protein structure determination, where it is used as concomitant tool to mass spectroscopy and nuclear mass resonance spectroscopy [73, 74]. Moreover, IR spectroscopy is adopted for reaction kinetic studies and quick and reliable determination of solution concentrations, in particular during reactions where traditional sampling has to be avoided due to safety issues [75, 76]. Generally, infrared spectroscopy of liquids is performed in transmission experiments, where an IR beam passes through a sample cell with defined thickness. Using transmission IR spectroscopy for concentration measurements during crystallization and precipitation experiments gives rise to two problems: First, this technique is not suited for in situ measurements in suspensions and therefore requires sampling, i.e. solid liquid separation; Second, the small sample thickness required for the accurate measurement of strongly absorbing aqueous samples cannot be realized. A solution to these disadvantages is the application of attenuated total reflection
2.2 In situ process analytical technologies

(ATER) in combination with infrared spectroscopy [77]. Attenuated total reflection Fourier transform infrared (ATER-FTIR) spectroscopy was initially used to study surface phenomena due to the low penetration depth of the IR light into the sample [78]. Recently, this technique was successfully applied to monitor the liquid phase during crystallization processes [79, 80, 81]. Moreover, ATER-FTIR was used in combination with an automated lab reactor to determine the solubility of succinic acid as a function of temperature [82]. ATER-FTIR can be even used to monitor the level of impurities during crystallization processes [75, 83]. This is of particular interest to the pharmaceutical industry, where small concentrations of similar molecules are often present during the crystallization step of new molecular entities which can have tremendous effects on the systems thermodynamics and kinetics. In this work, ATER-FTIR spectroscopy is used for in situ concentration measurements, e.g. to monitor changes of the absorption values of a dissolved substance in a suspension as a function of time. All ATER-FTIR measurements in this work were carried out using a ReactIR 4000 system from Mettler-Toledo (Schwerzenbach, Switzerland), equipped with a 11.75” DiComp immersion probe and a diamond as ATR crystal.

To obtain concentration values from absorbance data the law of Lambert-Beer is describing the relationship between the incident and transmitted radiation intensities in vibrational spectroscopy. It can be expressed as

\[ A = abc = \log_{10} \frac{I_0}{I} \]  

(2.2)

where \( A \) is the absorbance, \( a \) is the absorption coefficient, \( b \) is the effective path length, \( c \) is the sample concentration, \( I_0 \) is the radiation emitted by the spectrometer, and \( I \) is the transmitted radiation of the sample [84]. All IR spectra were recorded with the ReactIR 3.0 data acquisition software. Generally, univariate calibrations based on Eq. 2.2 describe the relationship between absorbance values and corresponding concentration sufficiently well for a single substance. Yet, the IR spectra of multiple component systems feature an increased complexity. In such cases, multivariate calibration techniques such as Partial Least Squares (PLS) have been applied to accurately determine solute concentrations of different compounds [85, 86, 87, 88].
2.2.2 FBRM

The Lasentec Focussed Beam Reflectance Measurement (FBRM) is a particle measurement technique based on the backward scattering of a laser beam upon incidence with solid material. The probe based FBRM technique allows for in situ particle analysis even at high solid concentrations. Fig. 2.8 displays the FBRM measurement principle. Inside of the FBRM probe a laser beam rotates at high velocity as depicted in Fig. 2.8(a). The optical set-up inside the probe focusses the laser beam in a point close to the sapphire probe window. Every time the laser hits a particle having larger dimensions than the wavelength of the laser (785 nm), the laser light is backscattered and detected by the instrument. Fig. 2.8(b) schematizes the pathway of the laser light crossing the particle. The backscattered light is processed by the device electronics which calculates the chord length of the detected particle as the product of the measured reflection time $\Delta t$ and the beam velocity $v_b$. The number of chord lengths is measured during a time interval determined by the user to yield the chord length distribution (CLD) as illustrated in Fig. 2.8(c).

So far, the FBRM technology has been used in a large variety of applications. The in situ analysis of shear stress on the formation and breakage of aggregates revealed insights in the rheology of SiO$_2$ [89] and cellulose suspensions [90]. The influence of different polymers and of the shear rate on flocculation in the in the pulp and paper [91, 92, 93, 94] and cement industries [95, 96] was analyzed using FBRM. In the field of biotechnology, FBRM was used to monitor the biomass concentration and aggregate size of plant suspension cultures [97, 98], to study the detachment and fractionation of biofilms as a function of shear stress [99], and to measure the growth of microorganisms [100] and yeast cells [101]. Furthermore, FBRM technology was applied to study emulsions [102], activated wastewater sludge [103] and CO$_2$ gas hydrate decomposition [104]. However, the largest number of publications are devoted to the application of FBRM in the field of crystallization. Besides the reoccurring subjects of modeling the CLD for a given particle population [105, 106] and the calculation of PSDs of the measured CLD signal [107, 108, 109, 110, 111, 112, 113, 114, 115, 116], the FBRM is mainly used to determine thermodynamics and kinetics
2.2 In situ process analytical technologies

Figure 2.8: Principle of Focussed Beam Reflectance Measurements: (a) probe details, (b) chord measurement (laser beam is perpendicular to the paper), (c) chord length distribution.

[117, 118, 119, 120, 121, 122, 123], to study polymorphic transformations [56, 124, 125, 126, 127] and for the control of crystallization processes [128, 129, 130]. The device used in this work was the Lasentec FBRM D600L (Mettler-Toledo, Greifensee, Switzerland).

2.2.3 In situ video microscope

In-situ high-resolution images have been taken using a Lasentec Particle Vision and Measurement (PVM) 800 probe (Redmond, USA). Six independent laser sources inside the PVM probe illuminate a fixed area at the probe tip. The backscattered light is focused on a CCD camera producing an image of 1760 μm × 1320 μm with a resolution of approximately 10 μm. The images yield time-resolved qualitative information about the average particle size and shape. Fig. 2.9 displays a typical image of suspended β L-glutamic acid particles.
Figure 2.9: In situ image of the needle shaped β polymorph of L-glutamic acid.

2.2.4 Raman spectroscopy

After the prediction of the Raman effect through quantum mechanic calculations of molecules by Heisenberg, Schrödinger and Dirac in the 1920’s, Raman and Krishnan described the corresponding experimental findings in 1928 [131], for which Raman was honored with the Nobel Prize in Physics two years later. The Raman effect is related to the inelastic scattering of incident light. If a light quantum $h\nu_L$ hits a molecule, the released energy gets elastically scattered in all directions with a high probability. This scattering process occurs at the frequency of the incident light quantum and is known as Raleigh scattering. However, at a lower probability also an inelastic, so-called Raman scattering process can occur, when the scattered energy quantum is $h\nu_L \pm h\Delta\nu$. The Raman scattered light is frequency-shifted with respect to the excitation frequency to lower or higher frequencies resulting in Stokes or Anti-stokes Raman scatter as illustrated in Fig. 2.10. Stokes scattering is by
2.2 In situ process analytical technologies

Figure 2.10: Jablonski energy diagram for the Stokes and Anti-Stokes Raman scattering.

far the stronger of the two processes for two reasons: Firstly, the photon is scattered at a lower energy during Stokes scattering compared to Anti-Stokes scattering; Secondly, the majority of a molecule population is in its ground vibrational state at room temperature, resulting in a higher probability of Stokes scattering. However, the magnitude of the frequency shift is independent of the excitation frequency. This “Raman shift” is therefore an intrinsic property of the sample offering a great potential in analytical chemistry. Commercial Raman spectrometers are available since the late 1950’s, but only the advent of Fourier-transform Raman spectrometers made this measurement technique interesting for standard analytical purposes. Generally, Raman spectroscopy is used nowadays as complementary technique to IR spectroscopy in the structure determination of new organic molecules. Besides, Raman spectroscopy allows contact-free analysis of temperature and composition of gaseous and liquid samples. Moreover, composition and crystal structure of solid state samples can be analyzed by Raman spectroscopy as well. Yet, Raman spectroscopy has the inconvenience that fluorescence might make it difficult or even impossible to record meaningful spectra [132].

In the framework of this thesis, Raman spectroscopy is used mainly to
characterize the polymorphic form and an eventual solid phase transformation of a solid material while it is suspended in a liquid. Wang et al. were among the first to apply in situ Raman spectroscopy to characterize a solvent mediated polymorphic transformation [133]. Since then, several authors have applied similar approaches to characterize solvent mediated polymorphic transformations of different systems such as D-mannitol [124], L-glutamic acid [56, 134], carbamazepine [135, 136], citric acid [47], calcium carbonate [137] and different active pharmaceutical ingredients (API) [138, 139]. In this thesis a RA 400 Raman spectrometer (Mettler-Toledo, Greifensee, Switzerland) was used, equipped with a 250 mW frequency stabilized laser diode at 785 nm and a thermoelectrically cooled detector. The spectrometer is connected via a fiber optic to a 5/8” ball type immersion Reaction RamanProbe from Inphotonics (Norwood, USA) with a wetted length of 330 mm that allows for acquisition within a spectral range from 200 to 3900 cm$^{-1}$ Stokes at a resolution of 3.6 cm$^{-1}$.

2.3 Ex situ analytical techniques

2.3.1 Particle counter and size analyzer

Accurate size characterizations of a particle population is not a trivial task. Commonly, instruments using laser diffraction are accepted as industrial standard to characterize the particle size distribution (PSD) of a given population. Due to the robustness of the principle, various authors have applied particle size analyzers based on laser diffraction also for the measurement of crystal growth kinetics [140, 141]. Yet, all diffraction instruments are based on a number of assumptions, e. g., the sphericity of the measured particles, to calculate the PSD from a measured diffraction pattern.

In this work, a Multisizer 3 from Beckman Coulter (Nyon, Switzerland) was employed to count the particles of a measured population and measure very accurately their volume at an unchallenged resolution. The device applies the electrical sensing zone or coulter method to measure PSDs. The measurement principles and basic assumptions of laser
diffraction and electrical sensing zone particle analyzers have been discussed in detail elsewhere [142, 143], and their accuracy was compared by different authors [144, 145]. The Coulter device determines the PSD of a population by measuring the volume of each particle which is then used to calculate the particle size of a volume equivalent sphere. Together with the total number of counted particles the size distribution of the volume equivalent spheres yields the PSD. This is a main advantage of this measurement technique compared to other particle analyzing techniques, since the mathematical model based on population balance equations (PBE) used in this work computes directly the PSD of volume equivalent spheres of the solid phase. Consequently, the experimental PSDs determined with the Multisizer 3 can be directly compared to the computed PSDs of the PBE model. Besides these advantages, two drawbacks of the electrical sensing zone technique can complicate PSD measurements under certain conditions: first, the measurement range is generally limited to 2 to 60% of the measurement orifice used; second, a suitable electrolyte has to be utilized throughout all measurements. The limited measurement range can be particularly disturbing when rather broad PSDs have to be characterized, because small particles will not be detected, while large particles tend to block the measurement orifice. The necessity to suspend the particles in an electrolyte solution can complicate measurements in organic solvents, when suitable electrolytes are hard to find, or change drastically the solubility of the solute compound. However, both inconveniences didn’t present any problem throughout this work, because the PSDs of all measurement samples were sufficiently narrow and a suitable electrolyte was found.

2.3.2 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique which is used to obtain thermodynamic parameters such as fusion temperature and melting enthalpy. The measurement principle of DSC is described in detail elsewhere [146]. In this work, a Mettler-Toledo DSC822e differential scanning calorimeter was used to determine the thermodynamic properties of L-glutamic acid and PDI 747, viz solid-liquid equilibrium, melting point, and melting enthalpy. About 10 mg of substance
were used for each experiment and the instrument was flushed with pure nitrogen during all experiments. The onset-temperature in the DSC diagrams was determined as described in the literature and was considered as the melting temperature of the substance [146].
Chapter 3

Precipitation of L-glutamic acid: determination of nucleation kinetics

This chapter presents a new procedure to determine the nucleation kinetics during the reactive precipitation of an organic substance. We have applied the new method to the pH-shift precipitation of L-glutamic acid upon mixing of an aqueous monosodium glutamate solution with hydrochloric acid. The induction time has been measured precisely in a stirred batch reactor by the combination of two in situ measurement techniques, namely ATR-FTIR spectroscopy and FBRM. It is shown, that ATR-FTIR is a suitable tool to measure the concentrations of the different L-glutamic acid ions and can be used to determine the starting time of the process, when the desired supersaturation is established in the reactor. The onset of particle formation is detected through FBRM. The precipitated polymorph is identified using in situ Raman spectroscopy and PVM. Finally, the induction time is used together with the independently measured growth kinetics to determine the nucleation rate.

This work was performed in collaboration with Lars Vicum and Martin Müller, and has been published elsewhere [49]
3.1 Introduction

Nucleation and growth are key mechanisms that determine the final crystal size, crystal size distribution and crystal shape of particulate products. Determining the rate of these processes is an important but difficult step in crystallization and precipitation process design and development. In particular the characterization of nucleation rates has proven to be challenging and up to now no standard measurement procedure could be established. Different techniques were proposed in literature, for example methods using combined particle counting and process time measurements [19, 20], MSMPR experiments in combination with particle size distribution measurements [8] or simultaneous nucleation and growth rate characterization from batch experiments using population balance modelling [15, 21, 22]. These methods are often time consuming and can require sampling, sample preparation and offline characterization, which can significantly reduce the measurement accuracy. The focus of this chapter is to establish a reliable and robust technique, which allows for the fast estimation of nucleation rates. For that purpose it is explored how on-line monitoring tools can be effectively exploited to measure the induction time, which in turn is used to calculate the corresponding nucleation rate. The precipitation of the metastable \( \alpha \)-form of L-glutamic acid through pH shift from a monosodium glutamate solution is employed to test the developed procedure. The induction time was measured as a function of initial supersaturation to derive the functional dependence of the nucleation rate on supersaturation. During the experiments in a semi-batch stirred tank reactor the temperature and impurity concentrations were tightly controlled and the mixing time was measured to ensure that the observed dynamics of particle formation was not influenced by mixing.
3.2 Experimental

3.2.1 Batch crystallizer set-up

A jacketed 2-liter borosilicate glass reactor with an inner diameter of 150 mm from LTS (Basel, Switzerland) was used in all experiments. The 4-blade glass stirrer had 45° inclined blades (with a diameter of 70 mm), was positioned 15 mm above the bottom of the reactor, and was operated always at 300 rpm. The temperature in the crystallizer was controlled using a Pt 100 and a CC 240 WL-3 thermostat from Huber (Offenburg, Germany). Fig. 3.1 shows the experimental setup together with the in situ measurement instruments. To optimize the quality of the data recorded in situ and to minimize clogging of the probe windows the position of the immersion probes was chosen in the zone of high fluid velocities, i.e. close to the bottom and near the reactor walls.

3.2.2 Materials and methods

L-glutamic acid monosodium salt monohydrate (≥98%, Sigma-Aldrich, Buchs, Switzerland), fuming hydrochloric acid solution (37-38%, L.T.Baker, Deventer, The Netherlands) and deionized water were used to precipitate L-glutamic acid by a pH-shift. L-glutamic acid has two known polymorphs [56, 66, 67]. For polymorph characterization purposes, pure β polymorph of L-glutamic acid has been purchased (>99%, Sigma-Aldrich, Buchs, Switzerland) and the metastable α-form has been produced as described by Cashell et al. [147]. The polymorphic form of the produced crystals was verified using Raman spectroscopy (RA 400, Mettler-Toledo, Greifensee, Switzerland) and scanning electron microscopy. The SEM samples were sputtered with 2 nm of platinum in high vacuum before being recorded with a Leo 1530 microscope (Zeiss/LEO, Oberkochen, Germany). Fig. 3.2 shows the Raman spectra together with scanning electron micrographs of both polymorphs. It can be readily observed that the Raman spectra exhibit several characteristic spectral differences as indicated by the arrows. It is worth noting, that the polymorphs can also be distinguished by the morphology since
3.2.3 Liquid phase monitoring using ATR-FTIR

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy has been applied successfully to monitor the liquid phase during crystallization processes [56, 79, 80, 81]. The ATR technique allows for the acquisition of liquid phase IR spectra in the presence of solid mate-
Figure 3.2: Raman spectra of the metastable $\alpha$ and of the stable $\beta$ form of L-glutamic acid. Characteristic spectral differences are highlighted for each form. Scanning electron micrographs of both polymorphs are also shown.

Material due to the low penetration depth of the IR beam on the surface of the ATR crystal which is generally in the order of 1 $\mu$m only [77]. All ATR-FTIR measurements in this chapter were carried out using a ReactIR 4000 system from Mettler-Toledo (Schwerzenbach, Switzerland), equipped with a 11.75” DiComp immersion probe and a diamond as ATR crystal.

3.2.4 Solid phase monitoring using FBRM

The Focused Beam Reflectance Method (FBRM) allows for in situ measurements of the chord length distribution (CLD) of the particle population even at high solid concentrations. It has been used for various purposes in the field of crystallization, such as the measurement of the solubility and of the metastable zone width [117], the estimation of crys-
3. L-glutamic acid nucleation kinetics

tallization kinetics [119], or the control of fines [128]. The principle of the measurement technique has been studied in detail elsewhere [148, 149]. In all experiments we have used a laboratory scale FBRM 600L from Lasentec (Redmond, USA).

### 3.3 L-glutamic acid speciation

L-glutamic acid has three functional groups, i.e. two acidic carboxylate and one basic amine group. Thus, in aqueous solutions the molecule reacts to yield four different charged and uncharged species through the following three protonation reactions:

\[
\begin{align*}
\text{Glu}^+ & \rightleftharpoons \text{Glu} + \text{H}^+ \\
\text{Glu} & \rightleftharpoons \text{Glu}^- + \text{H}^+ \\
\text{Glu}^- & \rightleftharpoons \text{Glu}^{2-} + \text{H}^+
\end{align*}
\]  

where $\text{H}^+$ is the $\text{H}_3\text{O}^+$ ion and $\text{Glu}^+$, Glu, $\text{Glu}^-$, and $\text{Glu}^{2-}$ indicate the protonated, neutral, dissociated, and twice dissociated form of L-glutamic acid, respectively. The neutral form is called free acid and is present as zwitterion, whereas $\text{Glu}^-$ is the glutamate ion. The relative amount of the four ions depends on pH. In this chapter, L-glutamic acid was precipitated by adding HCl to a monosodium glutamate, $\text{Na}^+\text{Glu}^-$, solution, since the monosodium salt has a high solubility (3.2 mol/l). An alternative would be to add NaOH to a solution of L-glutamic acid hydrochloride, whose solubility is about 2.7 mol/l at 20°C. The former allows for a wider range of initial supersaturation during pH-shift precipitation, hence it has been adopted. The solubility of the $\alpha$- and $\beta$-form of L-glutamic acid in water has been determined using ATR-FTIR spectroscopy as $7.4 \times 10^{-2}$ and $5.6 \times 10^{-2}$ mol/l at 25°C, respectively [56].

### 3.3.1 Speciation model

The speciation of L-glutamic acid in the presence of sodium and chloride ions can be determined as a function of pH by enforcing the following
dissociation equilibrium equations that correspond to the reactions of Eqs. 3.1 - 3.3 and to the autoprotonation of water (all equilibrium constants are at 25°C):

\[
K_\alpha = \frac{[Glu^-][H^+]}{[Glu^+]} = 6.46 \times 10^{-3} \quad (3.4)
\]

\[
K_\gamma = \frac{[Glu^-][H^+]}{[Glu]} = 5.62 \times 10^{-5} \quad (3.5)
\]

\[
K_b = \frac{[Glu^{2-}][H^+]}{[Glu^-]} = 2.14 \times 10^{-10} \quad (3.6)
\]

\[
K_w = [H^+][OH^-] = 1.001 \times 10^{-14} \quad (3.7)
\]

Here [Glu\(^+\)], [Glu\(^-\)], [Glu\(^{2-}\)], [H\(^+\)], and [OH\(^-\)] denote the concentrations of the glutamic acid species and of the H\(^+\) and OH\(^-\) ions, respectively. The values of the equilibrium constant \(K_\alpha\), \(K_\gamma\), \(K_b\), and \(K_w\) are taken from Lide [150]. Additionally, the following equations for the electroneutrality and the mass balance are also to be fulfilled:

\[
[H^+] + [Na^+] + [Glu^+] = [Cl^-] + [OH^-] + [Glu^-] + 2[Glu^{2-}] \quad (3.8)
\]

\[
[Glu_{tot}] = [Glu^+] + [Glu] + [Glu^-] + [Glu^{2-}] \quad (3.9)
\]

where [Na\(^+\)], [Cl\(^-\)], and [Glu\(_{tot}\)] represent the concentrations of Na\(^+\), Cl\(^-\), and the sum of all glutamic acid species, respectively. Eqs. 3.4 - 3.9 constitute a system of six equations in the six unknown concentrations \([Glu^+], [Glu], [Glu^-], [Glu^{2-}], [H^+], \) and \([OH^-]\) that can be solved for given values of the equilibrium constants and of the concentrations of \([Glu_{tot}],[Cl^-]\), and \([Na^+]\). The pH can be varied by changing the relative amounts of chloride and sodium ions. The equations above can be reduced by algebraic manipulations to a fifth order polynomial equation in \([H^+]\), under the assumption that the solution is ideal. The model given by Eqs. 3.4 - 3.9 is not accounting for changes in concentrations due to precipitation, thus it can be used exclusively to calculate the initial species concentrations and supersaturation before the onset of particle formation. Fig. 3.3 shows the concentrations of the different species as a function of the pH of the solution for a total ion concentration of 1 mol/l. When particle formation is also taken into account it has to be considered that the concentrations of the different L-glutamic
3. L-glutamic acid nucleation kinetics

Figure 3.3: Speciation of 1 mol/l L-glutamic acid as a function of pH, when precipitation is not allowed. Glu\(^+\), Glu, Glu\(^-\), and Glu\(^{2-}\) denote the concentrations of the protonated ion, the zwitterion, the glutamate ion, and the completely deprotonated ion of L-glutamic acid, respectively. Acid species are limited by their corresponding solubilities at equilibrium conditions. Under the conditions analyzed in this chapter only the free acid concentration reaches the solubility limit, therefore only one additional constraint has to be added when computing the equilibrium concentrations of the L-glutamic acid species:

\[ c_i^* \geq [Glu]; \quad (i = \alpha, \beta) \]  

(3.10)

where \( c_i^* \) is the solubility of the precipitate which can be either the \( \alpha \) or \( \beta \) polymorph. Using this constraint together with Eqs. 3.4 - 3.8 the equilibrium concentrations of the L-glutamic acid species can be calculated in combination with the following mass balance equation:

\[ [Glu_{tot}] = [Glu^+] + [Glu] + [Glu^-] + [Glu^{2-}] + [Glu_s] \]  

(3.11)
3.3 L-glutamic acid speciation

Figure 3.4: Speciation of 1 mol/l L-glutamic acid as a function of pH, taking into account the precipitation of α L-glutamic acid. Glu₅ denotes the concentration of precipitated free acid.

where \([\text{Glu}_s]\) is the equivalent concentration of the precipitate, i.e. its number of moles divided by the solution volume. Fig. 3.4 shows the speciation and the amount of precipitate of a 1 molar glutamate solution at equilibrium conditions as a function of the pH value.

3.3.2 Speciation monitoring using ATR-FTIR

The speciation of L-glutamic acid in aqueous solutions has been studied earlier using Raman and ATR-FTIR spectroscopy [151, 152]. The authors concluded that IR spectroscopy is a suitable technique to identify the different species of L-glutamic acid over a wide pH range. We performed similar experiments and recorded IR spectra of a 0.2 M Na-glutamate solution over a pH range from 1.5 to 12.2 at 25°C.
Figure 3.5: IR spectra of a 0.2 M Na-glutamate solution at different pH. The functional groups corresponding to characteristic spectral regions are indicated.

Fig. 3.5 shows the recorded IR spectra and highlights characteristic bands corresponding to the functional groups of the molecule. The figure clearly indicates that different characteristic bands dominate the spectrum at different pH values. The band at 1730 cm\(^{-1}\) can be attributed to the C=O stretching mode of the carboxylate group and therefore is only present in the spectra at pH values of 4.2 and below, i.e. when the acidic functional groups of L-glutamic acid are protonated [153]. On the contrary, the dominant bands at 1560 and 1404 cm\(^{-1}\) are strongest at high pH values and can be assigned to the asymmetric and symmetric carboxylate ion stretching vibrations, respectively. Additionally, the band at 1560 cm\(^{-1}\) is superimposed to a NH\(_2\) deformation band. Finally, the weak, sharp peak at 1451 cm\(^{-1}\) does not change over the whole pH range and can therefore be identified with a CH\(_2\) deformation mode, i.e. the "backbone" of the molecule.
3.3.3 Comparison model-experiment

The successful identification of different bands in the IR spectra allowed for the determination of the corresponding concentrations and for the comparison of the experimental data with the composition computed by means of the speciation model. In order to calculate concentrations from the absorbance data, simple two point calibrations were performed for each functional group using the peak area-to-baseline values and assuming the validity of Beer-Lambert's law:

\[ A = abc = \log_{10} \frac{I_0}{I} \]  

(3.12)

where \( A \) is the absorbance, \( a \) is the absorption coefficient, \( b \) is the effective path length, \( c \) is the sample concentration, \( I_0 \) is the radiation emitted by the spectrometer, and \( I \) is the transmitted radiation of the sample [84]. For the calibration of each functional group concentration the minimum absorbance value was associated with zero concentration and the maximum absorbance value was associated with the maximum concentration of each functional group. For the experiment presented in Figs. 3.5 and 3.6 with the nominal concentration of 0.2 mol/l, the equivalent maximum concentration of carboxylate groups was 0.4 mol/l since each completely protonated molecule features two carboxylate groups. Based on these calibrations the data shown in Fig. 3.5 can be used to determine the functional group concentrations as a function of the pH value as shown in Fig. 3.6. The figure also compares the experimental data with computational results based on the speciation model presented in section 3.3.1 (without precipitation). The functional groups concentrations have been calculated using the following equations, that account for the contribution of the different species to the concentration of the different functional groups:

\[ [\text{COOH}] = 2 [\text{Glu}^+] \]  

(3.13)

\[ [\text{CH}_2] = 2 [\text{Glu}_{\text{tot}}] \]  

(3.14)

\[ [\text{COO}^-] = [\text{Glu}] + 2 [\text{Glu}^-] + 2 [\text{Glu}^{2-}] \]  

(3.15)

\[ [\text{COO}^-] + [\text{NH}_2] = [\text{Glu}] + 2 [\text{Glu}^-] + 3 [\text{Glu}^{2-}] \]  

(3.16)
Figure 3.6: Comparison of the measured equivalent concentrations of different functional groups to the concentrations calculated by the ideal speciation model as a function of pH. The equivalent concentration of CH$_2$ is divided by two for improved readability.

It is worth noting, that in the figure the concentration values of the CH$_2$ group were divided by a factor of two to improve the readability of the diagram. It can be readily seen that the experimental data are in good agreement with the model predictions over the studied pH range. Therefore it can be concluded, that effects of changes in activity coefficients are not dominating for the conditions analyzed.

### 3.4 Induction time experiments

In this section, we present a new method for the accurate measurement of the induction time during pH-shift precipitation by combining two in
situ analytical techniques, namely ATR-FTIR spectroscopy and FBRM. After describing the experimental procedure in section 3.4.1, the particular advantages of the method are highlighted in section 3.4.2 and the experimental results for the L-glutamic acid precipitation are presented in section 3.4.3.

3.4.1 Measurement procedure

All induction time experiments were conducted at 25°C in the batch crystallizer described in section 3.2.1. A monosodium glutamate and a hydrochloric acid solution were initially prepared at equimolar concentration, and purified by filtration over a 0.22 μm filter (Millipore, USA). The sodium glutamate solution was filled in the reactor and the ATR-FTIR and FBRM measurements were started simultaneously. The measurement frequency of both instruments was chosen in such a way that several IR spectra and chord length distributions (CLD) were recorded during the expected induction period. After measuring initially a few IR spectra and CLDs to confirm the signal stability, an equimolar amount of hydrochloric acid in solution was added via a funnel with an attached hose to allow for a fast addition of the HCl solution without bubble formation which could corrupt the FBRM signal.

3.4.2 Measuring the induction time by combining ATR-FTIR and FBRM

The induction time of crystallization and precipitation experiments is generally defined as the period from the attainment of supersaturation to the detection of particles [154]. Therefore the measurement of induction times requires the determination of two points in time: first, the time when the initial supersaturation is established homogeneously throughout the reactor; second, the time when the first particles are detected. The precise and repeatable measurement of the induction time requires that the time needed to establish the initial supersaturation is short in comparison to the measured induction time. Additionally, the temperature and the impurity profile should be tightly controlled and the onset
Figure 3.7: Time evolution of the ATR-FTIR and FBRM signal at a supersaturation of $S_\alpha = 5.0$. The four phases of the experiment are discussed in detail in section 3.4.2.

of particle formation should be measured in a reproducible manner. In this chapter, two in situ measurement techniques for the liquid and the solid phase, namely ATR-FTIR spectroscopy and FBRM, were combined to measure the induction time as a function of supersaturation.

Fig. 3.7 shows the typical time evolution of the monitoring data during an experiment, i.e. the baseline integrated peak area of the peak at 1404 cm$^{-1}$ in the IR spectrum, which represents the concentration of the carboxylate groups, and the cumulative counts from 1 to 11 $\mu$m of the CLD measured by FBRM. It is worth noting, that the shown IR signal has not been calibrated since its qualitative information is sufficient for this study. The time evolutions in Fig. 3.7 can be divided into four phases. In phase 1, during the initial lag time of about 90 seconds both signals, ATR-FTIR and FBRM, remain constant. In phase 2, the HCl
solution is added to the reactor and a steep decrease of the ATR-FTIR signal can be observed, while the FBRM signal remains at zero. The decrease of the IR signal to one fourth of its initial value is due to the protonation of half of the carboxylate groups of the glutamate ion as well as to the dilution of the liquid phase. Yet, no particles have been formed and the FBRM counts remain at zero. It is worth noting, that the duration of phase 2 is within five to ten seconds identical for all induction time experiments in this chapter, since the total mass of both reactants was always the same. The point in time where the nominal supersaturation conditions have been attained everywhere in the reactor is clearly identified as the point, where the ATR-FTIR signal is again stable and constant. This is when the phase 3 begins, which is the induction period. However, identifying the end of the induction period based on the IR signal is impossible since the signal changes only slowly after the onset of particle formation. Therefore, one has to resort to the FBRM counts, which show a distinct increase upon particle detection as shown in Fig. 3.7. The time period between these two events is the induction time associated to the nucleation of L-glutamic acid crystals. It is worth noting, that the concentration level as measured by ATR-FTIR and therefore also the level of supersaturation remained constant during the induction time in all experiments. The fourth phase is characterized by the increase in FBRM counts and a decrease in the ATR-FTIR signal. The polymorphic form of the precipitate has been identified by in situ Raman spectroscopy and PVM as the metastable α form for the whole investigated supersaturation range. The results obtained for a supersaturation of $S_\alpha = 7.3$, when the induction time was 23 seconds are shown in Fig. 3.8. The PVM images show that the precipitated crystals have a prismatic shape, which is characteristic for the α polymorph. The indicated peaks in the recorded Raman spectrum are characteristic of the α form as well.
Figure 3.8: In-situ PVM images and characteristic Raman spectrum of α crystals precipitated at $S_\alpha = 7.3$. 
3.4 Induction time experiments

3.4.3 Induction time as a function of supersaturation

The experiment described in section 3.4.2 has been repeated at different initial supersaturation levels in order to measure the induction time as a function of supersaturation. Different supersaturation values are obtained by simply varying the concentration of Na-glutamate and hydrochloric acid in the two initial solutions. All experiments have been repeated at least twice and show a good reproducibility as presented in Fig. 3.9. As expected, the induction time decreases exponentially with increasing supersaturation. It is worth noting, that the experimental range for the initial supersaturation had to be limited to a maximum of $S_\alpha = 8.1$. For larger supersaturation values the experimental uncertainty increased significantly since the induction time is in the same order of magnitude as the mixing time. The set of averaged induction times will be used in the following section to calculate the nucleation rate.

![Figure 3.9: Measured induction times as a function of supersaturation.](image-url)
3.5 Nucleation rate determination

3.5.1 Calculation of the nucleation rate J

A general correlation relating the induction time \( t_i \) of the new phase formation with kinetics of the occurring fundamental mechanisms, i.e. nucleation and growth, was proposed by Kashchiev et al. [155]. The following equation was proposed for a polynuclear mechanism and 3D growth:

\[
t_i = \left( \frac{3\alpha_v}{\pi JG^2} \right)^{1/4}
\]

where \( t_i \) denotes the induction time, \( \alpha_v \) is the minimum volume fraction of the newly formed solid phase detectable with the measurement device, \( J \) is the nucleation rate, and \( G \) denotes the growth rate; both \( J \) and \( G \) depend on supersaturation. This relationship takes into account that particles are detected only after they have grown and the volume fraction of solid in the suspension exceeds a limit value. Consequently, the calculation of the nucleation rate \( J \) requires the knowledge of that detectable volume fraction \( \alpha_v \) and of the growth rate \( G \), which has to be measured independently. For the analytical device used in this chapter, i.e. the Lasentec FBRM, \( \alpha_v \) was estimated as \( 10^{-4} \) from earlier studies [156]. The growth mechanism of L-glutamic acid was identified as "birth and spread" and the kinetics of both L-glutamic acid polymorphs were measured by Kitamura [53]. We used the growth kinetics for the different faces proposed by Kitamura to calculate the following overall growth kinetics for the metastable \( \alpha \) form:

\[
G_\alpha = 1.6 \times 10^{-7} (S_\alpha - 1)^{5/6} \exp \left( \frac{-0.4}{S_\alpha - 1} \right)
\]

The validity of this growth rate correlation has been independently confirmed by seeded crystallization experiments at low supersaturation \( (S_\alpha \leq 4) \). However, crystal growth can become diffusion controlled at higher supersaturation levels. The diffusion controlled growth rate can be described by the following equation [6]:

\[
G_{dif} = \frac{Fk_{ij}}{3\rho_c} \Delta C
\]
where $F$ denotes the overall shape factor, $k_d$ is the mass transfer coefficient, $\rho_c$ is the crystal density, and $\Delta C$ represents the concentration difference between bulk and crystal surface. The mass transfer coefficient of the $\alpha$ polymorph $k_{d\alpha}$ can be predicted using the Sherwood correlation [6]:

$$k_{d\alpha} = \frac{D}{L} \left( 2 + 0.8 \left( \frac{\bar{e}L^4}{\nu^3} \right)^{1/5} Sc^{1/3} \right)$$

(3.20)

where $D$ denotes the diffusivity, $L$ the crystal size, $\bar{e}$ the average power input, $\nu$ the kinematic viscosity and $Sc$ the Schmidt number, i.e. $Sc = \nu / D$.

The comparison of the growth kinetics given in Eqs. 3.18 and 3.19 demonstrates that the growth of the $\alpha$ polymorph is never diffusion controlled and can be described by Eq. 3.18 in the studied supersaturation range. Therefore, the measured induction times will be used in the following section together with Eqs. 3.17 and 3.18 and the estimate for $\alpha_v$ to calculate the nucleation rate $J$ as a function of supersaturation.

### 3.5.2 Estimation of kinetic parameters

The values for the nucleation rate $J$ computed from Eqs. 3.17 and 3.18 can be used to determine kinetic correlations according to:

$$J(S) = A \exp \left( \frac{-C}{\ln^2 S} \right)$$

(3.21)

with the kinetic parameters $A$ and $C$ according to classical nucleation theory [6]. When plotting the calculated nucleation rates as $\ln(J)$ over $\ln^{-2}(S)$ as shown in Fig. 3.10, the experimental data can be interpolated by two straight lines corresponding to the mechanisms of homogeneous and heterogeneous nucleation. Based on the interpolations the following kinetic equations for homogeneous and heterogeneous nucleation were determined:

$$J_{Hom}(S_{\alpha}) = 1.3 \times 10^{27} \exp \left( \frac{-163}{\ln^2 S_{\alpha}} \right)$$

(3.22)

$$J_{Het}(S_{\alpha}) = 1.4 \times 10^{8} \exp \left( \frac{-10}{\ln^2 S_{\alpha}} \right)$$

(3.23)
3. L-glutamic acid nucleation kinetics

Homogeneous nucleation:

\[ A_{\text{Hom}} = 1.3 \times 10^2 \text{ m}^{-3} \text{ s}^{-1} \]

\[ C_{\text{Hom}} = 163 \]

Functional form:

\[ J = A \exp (-C (\ln S)^2) \]

Heterogeneous nucleation:

\[ A_{\text{Het}} = 1.4 \times 10^8 \text{ m}^{-3} \text{ s}^{-1} \]

\[ C_{\text{Het}} = 10 \]

Figure 3.10: Nucleation rate of α L-glutamic acid as a function of supersaturation (\( \ln(J) \) vs \( \ln^{-2}(S_\alpha) \)). The different nucleation regimes are highlighted and the corresponding kinetic parameters are indicated.

The overall nucleation rate is at any supersaturation level the sum of these two contributions, i.e.

\[ J(S_\alpha) = J_{\text{Hom}}(S_\alpha) + J_{\text{Het}}(S_\alpha). \]  

(3.24)

3.6 Conclusions

In this chapter a reliable and robust technique has been presented, which allows for the fast estimation of nucleation rates based on in situ induction time measurements. It could be shown that ATR-FTIR is a feasible technique to obtain precise information about the concentrations of the different L-glutamic acid species in solution. ATR-FTIR can be used to determine accurately the time interval needed to achieve mixing of the
solution in the pH-shift precipitation of L-glutamic acid and to detect the time, when supersaturation is homogeneously established throughout the reactor. Knowing the mixing time is of particular importance to be able to judge when the measurement technique is applicable, which is the case when the mixing time is short compared to the determined induction time. The experimental data presented prove that FBRM is a suitable technique to detect the onset of particle formation in a reproducible manner. However, it should be kept in mind that the detected onset of particle formation is strongly device and system dependent. An uncertainty in the estimation of the nucleation rate is introduced by the necessary assumption that the FBRM detects particles always beyond a minimum particle volume fraction. Furthermore, the nucleation rate estimation requires that the growth rate of the particles is known, which in turn requires additional experimental chapter and is another source of uncertainty. Nevertheless, the presented technique should allow for obtaining a reasonable estimate of the nucleation rate and it has clearly the advantage of being a rather fast method which works fully in situ and does not require sampling or sample preparation.
Chapter 4

Precipitation of L-glutamic acid: determination of growth kinetics

Seeded batch desuperaturation experiments were used for the fast and robust determination of α-L-glutamic acid growth kinetics independent of other phenomena like nucleation or agglomeration. In situ process analytical technologies, e.g., Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Focused Beam Reflectance Measurement (FBRM), and different ex situ analytical tools and population balance modeling were combined to determine the growth mechanism and estimate the kinetic parameters. The growth mechanism was established to be integration controlled by comparing the experimentally determined growth kinetics to a diffusion limited growth rate estimated using the Sherwood correlation. The experimental desupersaturation data was used together with population balance modeling and a non-linear least squares optimization to estimate the kinetic parameters for two integration controlled growth mechanisms, according to spiral dislocation growth (BCF) and surface nucleation (B+S) theories. The optimized kinetics equation for the B+S mechanism yielded smaller residuals compared to the BCF mechanism, suggesting a surface nucleation mecha-
nism which is in agreement with literature results and was validated using SEM photomicrographs. Finally, the experiments at higher initial supersaturation not used during the parameter estimation were utilized to validate the established growth rate correlation.

4.1 Introduction

Precipitation from solution includes several fundamental mechanisms, e.g. nucleation, growth and agglomeration, that determine the particle size distribution, shape and polymorphic form of the precipitated product. The ability to measure the kinetics of these mechanisms is of crucial importance for process design and development. Various methods can be found in literature to determine growth rate kinetics for crystallization processes: Besides single crystal methods, where the growth mechanism and kinetics of different crystal faces are usually determined by optical or atomic force microscopy (AFM) under different flow and supersaturation conditions, seeded batch multiparticle experiments are usually employed for process design purposes [8]. Recently, the use of in situ process analytical technologies (PAT) has become more and more frequent for the control and characterization of crystallization and precipitation processes. A closed loop control of a cooling crystallization process was implemented by monitoring liquid and solid phase using different in situ PATs to obtain a defined product crystal size [157]. During the cooling crystallization of monosodium glutamate various PATs were combined to estimate growth kinetics under these conditions [158]. A method using the Focussed Beam Reflectance Measurement (FBRM) for the direct estimation of crystal growth kinetics was proposed recently [127]. The combination of in situ concentration measurements and FBRM data with population balance modeling yielded the growth kinetics of paracetamol [125]. In the previous chapter, the nucleation kinetics of α L-glutamic acid during precipitation from monosodium glutamate solutions upon addition of hydrochloric acid was determined. This chapter focusses on the growth kinetics characterization of α L-glutamic acid under similar experimental conditions, i.e., when supersaturation is created via pH-shift. The growth kinetics char-
acterization used in this chapter combines the concept of seeded batch desupersaturation experiments described in literature [8] with different in situ PATs to monitor liquid and solid phase data to increase the accuracy of the determined growth kinetics. While the original method was based on the calculation of the first and second derivative of the desupersaturation curve and on the assumption of a non substantial change in crystal surface area during the experiment, the method presented here determines the growth rate parameters combining the complete liquid phase data of several experiments under different conditions with population balance modeling and an optimization routine. Since the absence of significant nucleation and agglomeration throughout the experiments is still a prerequisite for this method, the solid phase is monitored using FBRM during the experiments. Moreover, the final particle size distributions are compared to the model simulations to verify whether the assumed experimental conditions were met, thus validating the determined growth parameters. Additionally, scanning electron micrographs are used to check for undesired secondary effects.

4.2 Materials and methods

Monosodium glutamate monohydrate (≥ 98%, Sigma-Aldrich, Buchs, Switzerland), fuming hydrochloric acid solution (37-38%, L.T.Baker, Deventer, The Netherlands) and deionized water were used in all experiments. L-glutamic acid has two monotropically related polymorphs, the metastable α and the stable β form [66, 67]. In this chapter, the polymorphic purity of the solid fraction was characterized by X-ray powder diffraction, Raman spectroscopy and scanning electron microscopy as reported elsewhere [49, 56]. The solvent mediated polymorphic transformation from the metastable α to the stable β form was not observed in this chapter due to the relatively low temperature of 25°C.

4.2.1 Batch crystallizer set-up

A jacketed 500 mL borosilicate glass reactor with an inner diameter of 100 mm from LTS (Basel, Switzerland) was used throughout all experi-
Figure 4.1: Schematic of the 500 mL batch reactor combining the different in situ process analytical technologies of FBRM and ATR-FTIR spectroscopy. The figure is not in scale, particularly as far as the position of the probes is concerned.

ments. The 4-blade glass stirrer from LTS (Basel, Switzerland) with 45° inclined blades had a diameter of 50 mm, was positioned 10 mm above the reactor bottom, and was operated at 250 rpm to ensure a homogeneous dispersion of the crystals in the reactor. The temperature in the crystallizer was controlled using a Pt 100 together with a CC230 thermostat from Huber (Offenburg, Germany). Fig. 4.1 shows the experimental setup together with the in-situ measurement instruments used in this chapter. The position of the immersion probes was chosen in the zone of high fluid velocities, i.e., close to the impeller tips, to minimize clogging of the probe windows, thus optimizing the quality of the recorded data.
4.2.2 In situ monitoring using ATR-FTIR and FBRM

Combined attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and Focused Beam Reflectance Measurement (FBRM) have been recently applied successfully to monitor the liquid and solid phase during crystallization processes [49, 126]. ATR-FTIR allows for the acquisition of liquid phase IR spectra in the presence of solid material due to the low penetration depth of the IR beam into the liquid phase; FBRM is an established technique to record the chord length distributions (CLDs) of a suspended particle population even at high solid concentrations. The measurement principles of these techniques can be found in detail elsewhere [79, 148, 149].

In this chapter, ATR-FTIR was used to monitor the concentration of L-glutamic acid using the two bands in the IR spectra at 1224 cm\(^{-1}\) and 1408 cm\(^{-1}\) which correspond to the protonated and dissociated carboxylate groups of the molecule, respectively [152]. Two independent calibrations were performed for these two bands at 25 °C using a set of solutions with known L-glutamic acid concentrations applying the law of Beer-Lambert [49]. Fig. 4.2 shows both sets of calibrations, which exhibit high linearities with \(R^2\) values close to one. The differences in the determined concentrations based on these two calibrations was in the order of less than 4 %, which was considered to be acceptable. The supersaturation \(S\) was determined in this chapter by averaging the concentration values calculated with the two calibrations and dividing by the solubility of the metastable \(\alpha\) form \(c^*\):

\[
S = \frac{c_{1224} + c_{1408}}{2c^*}
\]  

(4.1)

where \(c_{1224}\) and \(c_{1408}\) denote the concentration values calculated with the IR bands at 1224 cm\(^{-1}\) and 1408 cm\(^{-1}\), respectively. The FBRM was used to monitor the CLD of the solid phase and to assure that no significant nucleation occurred over the course of the experiments as described in section 4.3.1. The FBRM was not used as a particle sizing device throughout this chapter, because the off line particle analyzing technique presented in section 4.2.3 was considered to be more accurate and
4. L-glutamic acid growth kinetics

Figure 4.2: Calibration data for the absorbance values at 1224 and 1408 cm$^{-1}$ at 25 °C, that correspond to the protonated and dissociated carboxylate group of the L-glutamic acid molecule.

the measured particle size distributions (PSDs) can be directly compared to simulation results. All measurements in this chapter were carried out using a laboratory scale FBRM 600L and a ATR-FTIR ReactIR 4000 system, which was equipped with a 11.75" DiComp immersion probe and a diamond as ATR crystal, both from Mettler-Toledo (Schwerzenbach, Switzerland).

4.2.3 Particle size analysis using electrical sensing zone method

The present study employs a Multisizer 3 from Beckman Coulter (Nyon, Switzerland), which applies the electrical sensing zone or coulter method to measure PSDs. The measurement principle of electrical sensing zone
4.3 Desupersaturation experiments

Desupersaturation experiments have been applied by various authors to determine overall growth kinetics parameters [159, 160]. In this chapter an improved method of batch desupersaturation experiments is being proposed, based on the accurate measurement of the seed PSDs and the desupersaturation curve, which are combined with population balance modeling and an optimization routine to determine the growth kinetics. The experimental technique as well as the limitations of this method are described in detail in section 4.3.1. An important prerequisite of the experimental technique is the production, preparation, and characterization of seed crystals, which is highlighted in section 4.3.2. Finally, the measurement results of the desupersaturation experiments are presented in section 4.3.3.

4.3.1 Experimental procedure

In this chapter, the determination of growth kinetics from seeded batch desupersaturation experiments is based on the accurate measurement of seed PSDs and solute concentrations. All desupersaturation experiments were conducted in the following way: first, a solution with an initial supersaturation was created by equimolar mixing of monosodium
Figure 4.3: Repeatability of two sets of desupersaturation experiments presented in this chapter. •, ○, ▲, and △ represent Run 5, 6, 11, and 12 of the set of experiments given in table 4.1, respectively. The different initial conditions are highlighted in the graph.

Glutamate and hydrochloric acid solutions in the temperature controlled reactor. Second, the in situ monitoring with ATR-FTIR and FBRM was started and afterwards a certain amount of dry seeds was fed to the reactor. All experiments were performed twice except for run 13, where particle formation on the probe window corrupted the measurement signal due to the high supersaturation. Fig. 4.3 shows measured desupersaturation curves for two repeated runs of experiments with different initial conditions. It can be readily observed that the repeatability of the presented experiments was highly satisfactory. The reproducibility of the PSD at the end of the desupersaturation experiments was similar and is not explicitly shown here, since the PSDs were not used for parameter estimation but mainly to check whether the necessary experimental conditions were met.
4.3 Desupersaturation experiments

Figure 4.4: FBRM data of Run 9. It can be easily observed that no significant nucleation occurred over the course of the experiment since the counts of small chords remain at a constant low level.

The method required that experimental conditions are chosen in such a way that particle growth is dominating the desupersaturation process and other competing mechanisms, e.g. nucleation, and mechanisms that alter the available crystal surface, e.g. agglomeration and breakage, are of negligible influence. For that purpose the experimental setup had to be designed in a favorable way and the operating conditions of experiments had to be chosen properly. In terms of operating conditions two process parameters, namely initial supersaturation and seed crystal surface, could be tuned to avoid other mechanisms than particle growth. Particle breakage for example was avoided using a glass impeller with rounded edges and stirring at the minimum stirrer speed while assuring complete dispersion of the crystal population in the solution. To confirm these requirements were met, the absence of significant nucleation was assured in this chapter by monitoring the CLDs using the FBRM
and SEM photomicrographs of the final particles were analyzed. A typical time evolution of a CLD during a desupersaturation experiment is shown in Fig. 4.4. It can be observed in this figure that the number of particle counts in the small size range is virtually constant which indicates that no nucleation occurred during the process. Similar behavior was observed in all experiments throughout in this chapter.

### 4.3.2 Preparation and characterization of seed crystals

The main objective of seed preparation was to obtain strain free, undamaged, and non-agglomerated crystals of the metastable α form of L-glutamic acid in three size fractions. Consequently, the seeds were produced by precipitation at low initial supersaturation and the different size fractions were obtained by wet-sieving the grown crystals. Monosodium glutamate and hydrochloric acid solutions of 0.5 mol/l concentration were prepared and purified by filtration with a 0.22 μm filter. Both solutions were mixed in a 2 L stirred batch reactor, where particle formation occurred. The precipitated crystals were wet-sieved using four sieves with nominal mesh sizes of 64, 125, 250, and 355 μm, respectively. Three seed fractions S, M, and L were obtained by collecting crystals triple sieved in the size ranges of 64 - 125 μm, 125 - 250 μm, and 250 - 355 μm, respectively. Crystals coarser than 355 μm or finer than 64 μm were discarded. Finally, the crystals of all three fractions were washed, filtered, and dried. The α polymorphic form of the seeds was confirmed using powder X-ray diffraction, SEM, and Raman spectroscopy as described elsewhere [49]. The PSDs of the three seed fractions were characterized using a Coulter Multisizer 3 as described in section 4.2.3. Fig. 4.5 shows the experimental PSDs obtained. Additionally, scanning electron micrographs were taken of the three seed fractions, which are depicted in Fig. 4.6. It is worth noting that the same magnifications were used for all images, thus allowing the direct comparison of the three fractions and making the size differences between them clearly visible. Moreover it can be readily observed, that all fractions consist mostly of single crystals and a few agglomerates.
4.3 Desupersaturation experiments

4.3.3 Experimental results

A series of experiments was conducted using different seed fractions, seed masses, and initial supersaturation values. The operating conditions of all experiments are listed in Table 4.1. The influence of changing either the seed fraction, seed mass or initial supersaturation on the measured desupersaturation curves is presented in Figs. 4.7, 4.8, and 4.9, respectively. In these figures the experimental data is shown together with simulation results which will be discussed in section 4.4. Experimental data are represented by symbols while simulation results are plotted as lines. It can be observed, that an increase of the total seed surface, either by reducing seed size as shown in Fig. 4.7 or by increasing seed mass as shown in Fig. 4.8, leads as expected to a faster decrease in supersaturation. Yet, the experimental desupersaturation curves show only slight differences for a change in seed mass, whereas an increase in crystal surface by seeding with smaller crystals results in a significantly
faster decrease of the desupersaturation curve. This can be related to the difference in available seed surface of the system, which is more significant in the experiments with different seed fractions. It is worth noting that for some of the experiments, in particular the two experiments with seed masses of 3 and 4 g shown in Fig. 4.8, the data acquisition was aborted somewhat to early, although the main decrease of supersaturation was monitored in all experiments. Moreover, it can be readily seen that during run 13, e.g., the experiment with an initial supersaturation of $S_0 = 3.86$ illustrated in Fig. 4.9, significant clogging of the probe window occurred which induced corruption of the in situ liquid phase data. The clogging of the ATR-FTIR probe window is probably due to the recessed position of the window at the tip of the probe. The change of IR spectra by particles on the probe window was even more pronounced in the lower band region of the IR spectra, where the appearance of several sharp bands indicated the clogging of the probe. Yet, upon cleaning of the probe window these additional bands disappeared and the measurement could be continued normally. At the end of each experiment the
### Table 4.1: Experimental conditions of the desupersaturation experiments and corresponding mean residual values of the two optimized growth correlations given in section 4.4.2. The seed fractions S, M, and L, correspond to small, medium and large average seed crystal size and the experimental PSDs and SEM photomicrographs are shown in Figs. 4.5 and 4.6, respectively.

<table>
<thead>
<tr>
<th>Run</th>
<th>$S_0$</th>
<th>Seed fraction</th>
<th>Seed mass</th>
<th>Mean residual (B+S)</th>
<th>Mean residual (BCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.94</td>
<td>S</td>
<td>2.0</td>
<td>$3.3 \times 10^{-3}$</td>
<td>$2.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>2</td>
<td>1.94</td>
<td>S</td>
<td>2.0</td>
<td>$4.4 \times 10^{-3}$</td>
<td>$3.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>3</td>
<td>1.94</td>
<td>M</td>
<td>2.0</td>
<td>$3.6 \times 10^{-3}$</td>
<td>$5.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>4</td>
<td>1.94</td>
<td>M</td>
<td>2.0</td>
<td>$2.3 \times 10^{-3}$</td>
<td>$4.6 \times 10^{-3}$</td>
</tr>
<tr>
<td>5</td>
<td>1.94</td>
<td>L</td>
<td>2.0</td>
<td>$1.2 \times 10^{-3}$</td>
<td>$2.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>6</td>
<td>1.94</td>
<td>L</td>
<td>2.0</td>
<td>$1.5 \times 10^{-3}$</td>
<td>$2.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>7</td>
<td>1.94</td>
<td>M</td>
<td>3.0</td>
<td>$1.3 \times 10^{-3}$</td>
<td>$1.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>8</td>
<td>1.94</td>
<td>M</td>
<td>3.0</td>
<td>$3.2 \times 10^{-3}$</td>
<td>$3.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>9</td>
<td>1.94</td>
<td>M</td>
<td>4.0</td>
<td>$5.1 \times 10^{-3}$</td>
<td>$5.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>10</td>
<td>1.94</td>
<td>M</td>
<td>4.0</td>
<td>$2.0 \times 10^{-3}$</td>
<td>$2.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>11</td>
<td>2.95</td>
<td>M</td>
<td>4.0</td>
<td>$5.0 \times 10^{-3}$</td>
<td>$5.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>12</td>
<td>2.95</td>
<td>M</td>
<td>4.0</td>
<td>$2.5 \times 10^{-3}$</td>
<td>$3.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>13</td>
<td>3.86</td>
<td>M</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mass of the grown crystals was determined after filtration, washing and drying. The yield of all experiments was within 92 and 97 % of the calculated masses. The PSD of the grown particles was measured after drying using the Multisizer 3. The measured PSDs corresponding to the experiments shown in Figs. 4.7, 4.8, and 4.9 are shown in Figs. 4.10, 4.11, and 4.12, respectively. These PSDs will be used to verify the assumptions for the experiments thus validating the growth rate parameters determined together with the population balance model presented in the following section.
Figure 4.7: Experimental and simulated desupersaturation curves of three experiments with different seed Fractions of S, M, and L = 2.0 g, respectively. All other experimental parameters were identical.

4.4 Parameter estimation

In the following the experimental results presented in the previous section are analyzed using a mathematical model based on the population balance equation (PBE). By combining the process model with an optimization algorithm the growth kinetics mechanism and parameters can be extracted from the experimental data. Section 4.4.1 describes the population balance based process model and the implemented parameter optimization algorithm based on a least squares minimization. The determination of the growth mechanism, the optimized growth parameters and the estimation of diffusional limitations is highlighted in section 4.4.2, whereas in section 4.4.3 the accuracy of the estimated parameters is evaluated with respect to the experimental results and the determined growth kinetics are compared to correlations given in literature.
4.4 Parameter estimation

Figure 4.8: Experimental and simulated desupersaturation curves of three experiments with different seed masses of M = 2.0 g, 3.0 g, and 4.0 g, respectively. All other experimental parameters were identical.

4.4.1 PBE model and optimization procedure

The population balance used in this chapter is based on the following hyperbolic partial differential equation:

$$\frac{\partial n(L, t)}{\partial t} + G \frac{\partial n(L, t)}{\partial L} = 0;$$

(4.2)

where \( L \) denotes the crystals size, \( t \) is the time, and \( n(L, t) \) is the number density of particles with the size \( L \) [161]. The crystal growth rate \( G \) is assumed to be size independent. This assumption could be validated by carefully designed experiments in this chapter as discussed in section 4.4.3. The molar concentration \( c \) of the solute in the liquid phase fulfills the following equation:

$$\frac{dc}{dt} = -\frac{1}{2M} k_a \rho G \int_0^\infty nL^2 dL,$$

(4.3)
Figure 4.9: Experimental and simulated desupersaturation curves of three experiments with different initial supersaturation values of $S_0 = 1.94$, 2.95, and 3.86, respectively. All other experimental parameters were identical.

where $M$ is the molar mass of the solute, $\rho$ and $k_a$ are the solid density and the surface shape factor of the $\alpha$ crystals, respectively. In this chapter, we have used the values of 1540 kg/m$^3$ for the solid density and $\pi$ for the surface shape factor, i.e., the rhombic shape of $\alpha$ L-glutamic acid crystals is approximated to a spherical one. The following initial and boundary conditions apply for the given PBE:

$$c(0) = c_0$$  \hspace{1cm} (4.4)  \\
$$n(t, 0) = \frac{J}{G}$$  \hspace{1cm} (4.5)

with $c_0$ being the initial concentration of the solute and $J$ the nucleation rate per unit volume. Both $G$ and $J$ depend on supersaturation, i.e., on the solute concentration $c$. The number-based PSD of the corresponding
seed fraction was defined as additional initial condition for $n(0, L)$. It is worth noting that no agglomeration or breakage is included in the PBE, since it is assumed that these mechanisms can be neglected during the growth experiments. Moreover, it is assumed that all nucleation events can be neglected in the simulation since this was validated experimentally using the FBRM during all growth experiments. Therefore the nucleation rate is defined as

$$J = 0$$  \hspace{1cm} (4.6)

The equation system above was solved using the discretization method developed by Kumar and Ramkrishna [162]. This technique combines the method of characteristics with the method of discretization and allows for the control of grid resolution and computational efficiency while overcoming problems of numerical diffusion and stability.
Figure 4.11: Experimental and simulated final particle size distributions of three experiments Run 4, 7, and 10 with different seed masses of $M = 2.0 \text{ g}, 3.0 \text{ g}, \text{ and } 4.0 \text{ g}$, respectively. All other experimental parameters were identical.

The optimization procedure used in this chapter is based on a non-weighted non-linear least squares algorithm which is described by

$$\min \sum_{m=1}^{N_e} \sum_{n=1}^{N_p} (S_{m,n} - \sigma_{m,n})^2$$

where $N_e$ is the number of experiments, $N_p$ the number of desupersaturation values per experiment, $S$ and $\sigma$ are the experimental and calculated supersaturation values, respectively.
4.4 Parameter estimation

**Figure 4.12**: Experimental and simulated final particle size distributions of three experiments (Run 10 +, Run 12 ▲, and Run 13 ○) with different initial supersaturation values of $S_0 = 1.94, 2.95, \text{ and } 3.86$, respectively. All other experimental parameters were identical.

### 4.4.2 Determination of the growth mechanism and parameters

Different crystal growth mechanisms have been proposed in the literature to characterize the growth kinetics of $\alpha$ L-glutamic acid during cooling crystallization. Tai et al. compared an integration-controlled BCF screw dislocation mechanism with the empirical two-step model and found the better description of the experiments using the BCF correlation [163]. Kitamura compared two integration-controlled mechanisms, the BCF and the surface nucleation based birth and spread (B+S) mechanism, and found that the B+S correlation only yielded meaningful results [53]. Since the growth mechanism is not unambiguously reported in the literature, the optimization algorithm was used together with the desuper-
saturation data to estimate the parameters for both surface integration-controlled growth mechanisms, B+S and BCF. It is worth noting that Run 13 could not be used for the parameter optimization due to the partly corrupted desupersaturation data, thus the growth parameters were estimated by using Run 1 to Run 12. The equations used to describe the B+S and BCF mechanism can be cast as follows [164]:

\[ G_{B+S} = A_{B+S}(S - 1)^{5/6} \exp(-B_{B+S}/(S - 1)) \] (4.8)

\[ G_{BCF} = C_{BCF} \left((S - 1)^2/D_{BCF}\right) \tanh(D_{BCF}/(S - 1)), \] (4.9)

where \( A_{B+S} \), \( B_{B+S} \), \( C_{BCF} \), and \( D_{BCF} \) are parameters (two for each model) that are estimated from experiments. The optimization of the growth rate parameters yielded the following values for the B+S mechanism:

\[ A_{B+S} = 2.8 \times 10^{-7} \text{m/s} \] (4.10)

\[ B_{B+S} = 0.56 \] (4.11)

and for the BCF correlation

\[ C_{BCF} = 2.2 \times 10^{-7} \text{m/s} \] (4.12)

\[ D_{BCF} = 1.17 \] (4.13)

Changing the initial values of the estimated parameters in the optimization procedure over several orders of magnitude always produced the same results, thus indicating a global optimum. It can be readily seen that in contrast to Kitamura’s results, also the BCF model yielded meaningful results. It is worth noting, that the mean residuals calculated for both estimated parameter sets exhibit values of the same order of magnitude between \(1.2 \times 10^{-3}\) and \(5.2 \times 10^{-3}\), e.g., both correlations are in fairly good agreement with the experimental values. Yet, comparing the mean residuals for the two growth models for each experiment, the B+S growth mechanism exhibits smaller residuals, e.g., a better agreement, for almost all experiments as shown in Table 4.1. This indicates that the B+S growth mechanism describes the observed growth behavior of
the experimental set more accurately, which is in agreement with the atomic force microscopy (AFM) studies reported by Kitamura, who did not observe any screw dislocation growth sites typically observed during BCF growth for this system [165]. Similarly, SEM photomicrographs of the crystal surface at high magnifications as the one shown in Fig. 4.13 feature a rough crystal surface which is evenly distributed and no typical kink and step sites can be seen as described BCF theory elsewhere [166], thus suggesting surface nucleation as observed for the B+S mechanism. Consequently, the kinetics described by the B+S mechanism will be used in the following sections.

In a second step, we checked whether the growth kinetics was influenced by diffusional limitations. Therefore, the mass transfer coefficient $k_d$ was predicted using the Sherwood correlation [6]:

$$k_d = \frac{D}{L} \left( 2 + 0.8 \left( \frac{\overline{e} L^4}{\nu^3} \right)^{1/5} S_c^{1/3} \right)$$  \hspace{1cm} (4.14)

where $D$ is the diffusivity, $L$ the crystal size, $\overline{e}$ the average power input, $\nu$ the kinematic viscosity and $S_c$ the Schmidt number, i.e. $S_c = \nu/D$. With values of $D = 2 \times 10^{-9}$ m$^2$/s, $L = 2 \times 10^{-4}$ m, $\overline{e} = 5.9 \times 10^{-2}$ W/kg, and $\nu = 1 \times 10^{-6}$ m$^2$/s the mass transfer coefficient $k_d$ from equation 4.14 is $2.2 \times 10^{-4}$ m$^2$/s. Diffusion controlled crystal growth rates can be calculated using the following equation [6]:

$$G = k_d \frac{k_a}{3k_v \rho_c} \Delta c$$  \hspace{1cm} (4.15)

where $\rho_c$ is the crystal density, $\Delta c = c - c^*$ is the concentration based supersaturation, and $k_a$ and $k_v$ denote the surface and volume shape factors, respectively. Yet, the experimental growth rates were determined to be at least one order of magnitude lower than the diffusion limited growth rates computed with the mass transfer coefficient predicted by the Sherwood correlation. Therefore it was concluded, that the growth of $\alpha$ L-glutamic acid is controlled by the surface integration of new molecules in the crystal lattice under the conditions studied in this chapter.
4.4.3 Comparison model-experiment

The accuracy of the model predictions and the validity of the initial assumptions can be evaluated by comparison of the computed model results to the experimental liquid and solid phase data, e.g., the desupersaturation curve and the final PSD. It is worth noting that the desupersaturation curve is a time-resolved experimental information yielding several measurement information over the course of an experiment while the final PSD is an integral one. However, the desupersaturation data is also an integral information with respect to the material balance as described by Eq. 4.3, since the data describes not the solid phase responsible for the solute consumption over time. Consequently, besides the accurate information about the crystal surface at the beginning of the experiment we need the additional assumption, that the solid phase present at the beginning of the experiment is altered mainly by growth during the process. This assumption is validated by comparison of sim-
ulated and experimental final PSD for each experiment. Yet, although the final PSD is kinetics independent in a seeded batch experiment since it is only defined by the mass balance, it contains valuable information whether and to what extend undesired phenomena like nucleation or agglomeration occurred during the experiment.

First, let us consider the experimental and simulation results obtained by using the different seed fractions, shown in Figs. 4.5 and 4.6, but otherwise identical operating conditions. The desupersaturation curves and final PSDs corresponding to the different seed fractions are shown in Figs. 4.7 and 4.10, respectively. It can be observed in Fig. 4.7 that the computed desupersaturation curves are in good agreement with the experimental data which is also reflected by the low values of the mean residuals given in Table 4.1. If we compare the final PSDs for these three experiments shown in Fig. 4.10, it is worth noting that all PSDs computed for Run 1, 4, and 5 agree well with the experimental results but show the clear trend of increasing agglomeration intensity with decreasing particle size, i.e., increasing number of particles. This conclusion is supported by SEM micrographs of grown crystals at the end of Run 1 as shown in Fig. 4.14, where several agglomerates of single crystals can be seen. It is worth noting that despite the different degrees of agglomeration in Run 1, 4, and 5, the model results agree quite well with the experimental liquid and solid phase data.

The experimental and modeling results obtained using different seed masses of fraction M of either 2, 3, or 4 g, are illustrated in Figs. 4.8 and 4.11. Similar to the desupersaturation curves with different seed fractions, also the computed profiles for different seed masses are in very good agreement with the experimental values, although the differences between the three runs are rather small. The small differences between the three processes are also evident in Fig. 4.11 where the final PSDs are shown. While the computed PSDs are very similar and only slightly shifted versus each other along the abscissa, the experimental PSDs exhibit some slight broadening which can be explained with different degrees of agglomeration. It is worth noting that while Run 4 shows a PSD shifted to slightly larger sizes the PSDs of Run 7 and 10 are almost identical, which cannot be due to growth only but indicates stronger agglomeration during Run 10 due to a higher load of seed mass. This
results in small differences between simulated PSDs and experimental results. When comparing the model prediction quality for the solid and liquid phase properties, i.e., the supersaturation in Figs. 4.7 and 4.8 and the PSDs in Figs. 4.10 and 4.11, it can be clearly observed that supersaturation data can be predicted with higher accuracy. Therefore it can be concluded, that the time-resolved liquid phase data is not significantly affected by secondary phenomena such as agglomeration. Consequently, the desupersaturation data is suited for a robust method to determine growth kinetics parameters. However, the final PSDs are indispensable to check whether and to what extend the basic conditions of minimizing concurrent phenomena were respected during the experiments. Finally, in combination with the experiments using different seed fractions it can be concluded, that size dependent crystal growth, a phenomena observed for other systems [167], cannot be observed in the size range of the three seed fractions used.
Finally, let us compare the model and experimental results at different supersaturation values, particularly for Run 13 which was not used for the parameter optimization procedure and therefore the corresponding model results can be considered as predictive. The computed and experimental results of the desupersaturation curve and the final PSD data are shown in Figs. 4.9 and 4.12, respectively. Similar to the experiments with varying crystal surface presented above, the model results for different initial supersaturation are in very good agreement to the experimental desupersaturation curves. It is worth noting that this holds as well for the model prediction at an initial supersaturation of $S_0 = 3.86$ that corresponds to the experimental conditions of Run 13 where the experimental values are not influenced by probe clogging. An interesting feature of the system can be observed by closely comparing the course of the different experiments shown in the inset of Fig. 4.9. It can be readily observed, that the measured supersaturation values of Run 13 drop below the values of both other experiments. This crossover is also predicted by the simulation results. The computed desupersaturation curves of Run 10 and 12 show also a crossover, which is yet too small to be verified experimentally. The reason for the crossing over of the desupersaturation curves is related to the significant change of available crystal surface over the course of the experiment. Fig. 4.15 shows the evolution of the crystal surface for the three initial supersaturation values computed with the model. The initial crystal surface is the same in all three cases since the same seed fraction and seed mass were used. Yet, due to the higher crystal growth rates at higher supersaturation the available crystal surface area increases faster in Runs 12 and 13 when compared to Run 10. This results in a higher mass deposition rate and leads ultimately to the crossover behavior observed experimentally. The final PSDs of Run 10, 12, and 13 are shown in Fig. 4.12. Again, the experimental PSDs indicate some agglomeration resulting in broader distributions compared to the PSDs computed with the model. The trend of larger particles with increasing initial supersaturation observed in the experiments is captured well by the model.

Unlike cooling crystallization, where the driving force of the process is induced by a change in temperature the supersaturation was created in this chapter by a chemical reaction. Thus, the growth kinetics of $\alpha$
L-glutamic acid were studied at constant temperature. Yet, the temperature dependence of growth kinetics could be easily measured by repeating the experiments at different temperatures. So far, no overall growth kinetics for α L-glutamic acid at 25°C have been reported in literature. Yet, overall growth kinetics at 15 and 45°C [168, 56] have been published for this system which are plotted together with the current data at 25°C in Fig 4.16. It is worth noting that the comparison of the kinetics at different temperatures follows the expected trend of increasing growth kinetics with increasing temperatures. Moreover, the kinetics measured at 25 and 45°C both employ the kinetic equation of the B+S growth mechanism and the values of the pre-exponential factor $A_{B+S}$ are similar, differing only by about 10%. This is consistent with the temperature dependence of the B+S growth equation, where the factor $A_{B+S}$ is constant while the exponential factor $B_{B+S}$ is a function of temperature following the Arrhenius equation.
4.5 Conclusions

Figure 4.16: Comparison of the growth kinetics of α-L-glutamic acid measured in this chapter at 25°C with kinetics found in literature. The solid, dashed and dotted lines represent the growth kinetics at 15, 25, and 45°C, respectively.

4.5 Conclusions

An advanced method for the fast and robust measurement of growth rate kinetics is presented in this chapter. The method is based on two in situ process analytical technologies, namely ATR-FTIR spectroscopy and FBRM to monitor the liquid and the solid phase during seeded batch desupersaturation experiments. Additionally, a Coulter particle analyzer employing the electric zone sensing method is used to characterize the initial PSD of the different seed fractions. The experimental requirements for an independent growth kinetics determination, i.e., the absence of other mechanisms such as nucleation, agglomeration and breakage, are checked using in situ FBRM, an off line Coulter particle analyzer and scanning electron microscopy. It could be shown, that the presented char-
characterization method is robust also in case of occurring agglomeration. For the first time, the overall growth kinetics were determined independently for α L-glutamic acid at 25°C when the supersaturation is created by pH-shift. The growth mechanism was identified as birth and spread in agreement with literature. The comparison of the determined growth kinetics to correlations at different temperatures proposed by other authors has shown reasonable agreement.
Chapter 5

Precipitation of L-glutamic acid: Process modeling

In this chapter a mathematical model based on the population balance equation is developed to simulate the precipitation process of L-glutamic acid via pH shift. In a first step, the induction time data and the growth rate correlation determined experimentally in the previous chapters are used to recalculate the primary homogeneous and heterogeneous nucleation rates of L-glutamic acid. These nucleation and growth kinetics are then combined with a population balance model. The comparison of experimental and model results can be used to obtain an improved estimate of the so far unknown value of the detectable volume fraction $\alpha_v$ as well as to check whether other fundamental mechanisms influence the final PSD, i.e. agglomeration. Finally, the determined value of $\alpha_v$ is used to calculate optimized nucleation kinetics which are used with the population balance model to predict the influence of the most important process parameter, i.e., the initial supersaturation, on the particle size distribution (PSD) of the final product.
5.1 Process model

A mathematical model based on the population balance equation can be used to simulate the precipitation process of L-glutamic acid via pH-shift in aqueous solutions by combining the model and the nucleation and growth rate correlations determined in the previous chapters. The model describes the time dependent particle size distribution $n(t, L)$ which follows the general population balance equation [161]:

$$\frac{\partial n}{\partial t} + G \frac{\partial n}{\partial L} = 0; \quad (5.1)$$

where $L$ is the crystals size and $G$ is the mean crystal growth rate of L-glutamic acid. It is worth noting, that in the previous sections two different correlations for the crystal growth kinetics of L-glutamic acid were given. The first correlation given in Eq. 3.18 was estimated from the growth kinetics of different crystal faces reported in literature [53] whereas the second correlation given in Eqs. 4.8, 4.10, and 4.11 has been measured using seeded batch desupersaturation experiments and is considered as more accurate. Thus, the crystal growth kinetics are described by the following equation:

$$G_{B+S} = 2.8 \times 10^{-7} (S - 1)^{5/6} \exp(-0.56)/(S - 1) \quad (5.2)$$

The molar concentration $c$ of the solute in the liquid phase fulfills the following equation:

$$\frac{dc}{dt} = -\frac{1}{2M} k_a \rho G \int_0^\infty nL^2 dL, \quad (5.3)$$

where $M$ is the molar mass of the solute, $\rho$ and $k_a$ are the solid density and the surface shape factor of L-glutamic acid, respectively. As described in section 4.4.1, the values of 1540 kg/m$^3$ and $\pi$ were used for the solid density and the surface shape factor, respectively. The following initial and boundary conditions apply:

$$c(0) = c_0, \quad (5.4)$$
$$n(0, L) = 0, \quad (5.5)$$
$$n(t, 0) = \frac{J}{G} \quad (5.6)$$
5.2 Comparison of simulations and experimental results

with $c_0$ being the initial concentration of the solute and $J$ the nucleation rate per unit volume. It is worth noting, that the nucleation rate correlations given in Eqs. 3.22 and 3.23 were calculated with inaccurate estimate for the L-glutamic acid growth kinetics. Using the measured growth rate correlation which is supposed to be more accurate and is given in Eq. 5.2 together with an estimate for the detectable volume fraction of $\alpha_v = 1 \times 10^{-5}$ the following kinetics for the primary homogeneous and heterogeneous nucleation of L-glutamic acid can be calculated:

\[
J_{Hom} = 1.9 \times 10^{26} \exp\left(\frac{-162}{\ln^2 S}\right) \tag{5.7}
\]

\[
J_{Het} = 2.2 \times 10^7 \exp\left(\frac{-9.6}{\ln^2 S}\right) \tag{5.8}
\]

The overall nucleation rate is at any supersaturation level the sum of these two contributions, i.e.

\[
J(S) = J_{Hom} + J_{Het}. \tag{5.9}
\]

The upper system of equations was solved employing a method developed by Kumar and Ramkrishna [162]. It is based on a combination of the method of discretization and the method of characteristics yielding improved computational efficiency while overcoming problems of stability and numerical diffusion.

5.2 Comparison of simulations and experimental results

Precipitation experiments with an initial supersaturation of $S = 3.5$ were performed to validate the measured nucleation and growth kinetics by comparing the final PSDs of the experiment with the simulation results. Fig. 5.1 shows the experimentally obtained PSD together with two simulated PSDs which were obtained using the PBE model with two different nucleation rate equations. These nucleation rate equations
were calculated from the induction time data and the growth rate kinetics presented employing two different values for the detectable volume fraction $\alpha_v$. It can be readily observed, that the model employing nucleation rates given in Eqs. 5.7 and 5.8 calculated with the estimated value for $\alpha_v = 1 \times 10^{-5}$ results in a PSD that features a significantly larger average particle size than obtained in the experiment. Since no values for $\alpha_v$ are reported in literature for the measurement device used and the system studied, a new value for $\alpha_v = 4 \times 10^{-3}$ could be estimated by comparing the experimental and simulated PSDs. It is worth noting that $\alpha_v$ influences the pre-exponential factor of the calculated nucleation kinetics in a linear way, but estimates larger than $10^{-2}$ seem to be unrealistic. The new estimate for $\alpha_v$ results in the following correlations for the homogeneous and heterogeneous nucleation rates for L-glutamic
Figure 5.2: SEM photomicrograph of agglomerated L-glutamic acid particles precipitated via pH-shift at an initial supersaturation $S_0 = 3.5$. The number of primary particles in the agglomerate and the size of the agglomerate explain the deviation between simulated and experimental final PSDs, since the simulation includes only nucleation and growth phenomena.

$$ J_{Hom} = 7.6 \times 10^{28} \exp \left( \frac{-162}{\ln^2 S} \right) $$

$$ J_{Het} = 8.6 \times 10^9 \exp \left( \frac{-9.6}{\ln^2 S} \right) $$

However, the shape of the experimental PSD shown in Fig. 5.1 is significantly broader and not in agreement with the simulated PSD for the new value of $\alpha_v$. This indicates the presence of secondary mechanisms other
than nucleation and growth during the precipitation process which are not captured by the model. It is worth noting, that the largest crystals observed experimentally are about twice the size of the largest simulated particles. Such a difference could be explained by particle agglomeration. To check the validity of this assumption, SEM photomicrographs of the final L-glutamic acid particles were analyzed. Fig. 5.2 shows typical L-glutamic acid agglomerates in the precipitated product particles at an initial supersaturation of $S_0 = 3.5$. It can be readily observed, that each agglomerate consists of several single particles thus leading to a significant increase in particle size. Thus it can be concluded that agglomeration effects lead to the differences between the experimental and the simulated PSDs, since the model includes nucleation and growth phenomena only. It is worth noting, that the agglomeration effects observed during unseeded precipitation experiments are in agreement with the agglomeration behavior observed during the growth characterization experiments in chapter 4.

However, the experimental data is not sufficient to characterize the kinetics of agglomeration. Further experiments have to be performed to characterize the agglomeration kinetics of L-glutamic acid during pH-shift precipitation. One possibility to characterize agglomeration is on the basis of seeded batch desupersaturation experiments with concurrent crystal growth and agglomeration. Despite the concurrent phenomena, the agglomeration kinetics can be determined rather easily employing different experimental conditions with respect to seed mass and initial supersaturation, since the concomitant growth kinetics have been already determined. Other possibilities to determine the agglomeration kinetics have been proposed in literature for salicylic acid [169], m-chloronitrobenzene [170] and sodium chloride [171]. However, this is not in the scope of this work, although it has a significant influence on the final PSD.

5.3 Process simulation

The combination of the population balance equation model described in the previous section with the kinetics equation for size independent
growth and the corrected correlations for homogeneous and heterogeneous nucleation can now be used to simulate the process for different initial conditions. The effect of initial supersaturation on the final PSD was simulated in the supersaturation range of the induction times experiments, that were performed to determine the nucleation rate in section 3.5. The initial supersaturation value $S_0$ is a key experimental parameter for precipitation processes, since according to classical nucleation theory the kinetics of primary nucleation depend in a highly non-linear way on supersaturation, which is captured in the nucleation kinetics determined for L-glutamic acid that are illustrated together with the measured growth kinetics in Fig. 5.3. The simulated final PSDs are shown with the corresponding initial supersaturation values in Fig. 5.4. The simulated PSDs in the range of $S = 2.8$ to 5.7, which are dominated by heterogeneous nucleation are plotted as dashed lines, whereas the PSDs
5.4 Conclusions

In this chapter, a process model based on population balance modeling has been presented, which employs the determined nucleation and growth kinetics to simulate the precipitation of L-glutamic acid via pH-
shift. It could be shown, that the comparison of simulated and final PSDs can be used for two different purposes. Firstly, it allowed for the determination of the value for $\alpha_v = 4 \times 10^{-3}$, i.e., the detectable volume fraction used in the previous chapter 3 to calculate nucleation rates from induction time data. This is a valuable information since the order of magnitude of $\alpha_v$ was based so far on estimates obtained from other model systems, e.g., glass and ceramic spheres. Secondly, the comparison of experimental and simulated PSDs gives evidence whether the precipitation process can be described exclusively by nucleation and growth, or whether secondary mechanisms have to be taken into account. Since the experimentally obtained L-glutamic acid PSD featured significantly larger particles than simulated by the model based on nucleation and growth, it was assumed that agglomeration effects could be responsible for the larger particles. This assumption was validated using SEM photomicrographs. However, further experimental data is necessary to determine agglomeration kinetics in order to obtain a complete process model of L-glutamic acid precipitation. The corrected correlations describing homogeneous and heterogeneous nucleation were used together with the measured growth kinetics and the process model to simulate the dependence of the final PSD on the initial supersaturation during L-glutamic acid precipitation.
In this chapter the application of four different in situ analytical techniques to monitor the solvent-mediated polymorphic transformation of L-glutamic acid is presented. Focused Beam Reflectance Measurements (FBRM) and Particle Vision & Measurement (PVM) have been used to track the chord length and morphology of the crystals over the course of the transformation. The polymorphic forms present have been monitored using Raman spectroscopy while Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy has been used to measure the liquid phase concentration profile. The combination of the different

This work was performed in collaboration with Davide Bonalumi, Lars Vicum, and Martin Müller, and has been published elsewhere [56]
in situ data was used to identify the fundamental phenomena of nucleation and growth that govern the process. Moreover, the measurement data was combined with a mathematical model based on population balance equations and the fundamental equations describing the kinetics of nucleation and growth of both polymorphs. This combination allowed for the estimation of the characteristic nucleation and growth rates of the two polymorphic forms, while the dissolution process of the metastable polymorph was estimated using a Sherwood correlation. Finally, the experimental results obtained with different initial conditions and their simulation allowed for the validation of the population balance model and for a deeper understanding of the transformation process.

6.1 Introduction

Polymorphism is the ability of a substance to crystallize in different crystal modifications, each of them having the same chemical structure but different in the stacking of atoms or molecules in the crystal lattice. Several physical properties are affected by polymorphism, such as stability, solubility, melting temperature, hygroscopicity, chemical reactivity, and rate of dissolution. Due to differences in stability, the solid-state transformation of one polymorph into another can occur [172]. The solvent-mediated polymorphic transformation is induced by the difference in solubility of the polymorphs. In recent years, there has been a growing interest in monitoring and controlling the solid phase transformation during crystallization and precipitation processes, particularly in the pharmaceutical and fine chemical industries [173, 174]. Different offline analytical techniques have been used to characterize the polymorphs obtained during crystallization, namely X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA). These techniques require sampling and are therefore not suitable for the in situ monitoring of formation and transformation of polymorphs during the process. Recently, Raman spectroscopy has been successfully applied to monitor the polymorphic transformation in situ by different research groups [133, 134, 175]. Moreover, in situ Raman spectroscopy has been combined with a population balance model
to estimate the polymorph transformation kinetics of L-glutamic acid [50]. In this chapter, we want to combine the different in situ analytical techniques mentioned above to monitor the liquid and the solid phase during the polymorphic transformation of the metastable α form of L-glutamic acid into the stable β form at 45°C. The experimental data are then used together with a population balance model accounting for the fundamental phenomena to estimate the relevant parameters in the relationships for crystal nucleation and growth. Finally, the accuracy of the model is assessed.

6.2 Experimental

6.2.1 Materials and methods

The stable β polymorph of L-glutamic acid (>99%, Sigma-Aldrich, Buchs, Switzerland) and deionized water were used for all experiments. To produce pure metastable α-form, an aqueous L-glutamic acid solution with a concentration of 48 g/kg solvent was cooled from 80°C to 45°C at a rate of 1.5 K/min. Nucleation started only after 45°C had been reached, and soon after nucleation the suspension was filtered, washed and dried. The polymorphic form was verified using X-ray powder diffraction and scanning electron microscopy. SEM samples were sputtered with 2 nm of platinum in high vacuum before being recorded with a Leo 1530 microscope (Zeiss/LEO, Oberkochen, Germany). For both polymorphs, Figs. 6.1 and 6.2 show the characteristic XRD patterns and scanning electron micrographs, respectively. It is worth noting, that crystals of the α form are prismatic, whereas those of the β form are needle-like.

6.2.2 Batch Crystallizer Setup

A jacketed 2-liter borosilicate glass reactor with an inner diameter of 150 mm from LTS (Basel, Switzerland) was used as crystallizer in all experiments. The 4-blade glass stirrer had 45° inclined blades (with a
Figure 6.1: X-ray powder diffraction patterns of the metastable α and the stable β form of L-glutamic acid.

diameter of 70 mm), was positioned 15 mm above the bottom of the reactor, and was operated at 300 rpm. The temperature in the crystallizer was controlled using a Pt 100 and a CC 240 WL-3 thermostat from Huber (Offenburg, Germany). Fig. 6.3 shows the experimental setup together with the four in situ measurement instruments. To optimize the quality of the data recorded in situ and to minimize clogging of the probe windows the position of the immersion probes was chosen in the zone of high fluid velocities, i.e. close to the bottom and near the reactor walls.

6.2.3 Concentration measurement using ATR-FTIR spectroscopy

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy can be successfully applied to monitor the liquid phase during
crystallization processes [79, 80, 81]. The ATR probe allows for the acquisition of liquid phase IR spectra in the presence of solid material due to the low penetration depth of the IR beam which is generally in the order of 1 μm only [77]. All ATR-FTIR measurements in this chapter were carried out using a ReactIR 4000 system from Mettler-Toledo (Schwerzenbach, Switzerland), equipped with a 11.75" DiComp immersion probe and a diamond as ATR crystal.

**ATR-FTIR calibration**

The law of Lambert-Beer is the fundamental equation describing the relationship between the incident and transmitted radiation intensities in vibrational spectroscopy. It can be expressed as

\[ A = abc = \log_{10} \frac{I_0}{I} \]  

(6.1)
Figure 6.3: Schematic of the 2-L batch crystallizer combining the different in situ process analytical technologies of FBRM, PVM, ATR-FTIR and Raman spectroscopy

where $A$ is the absorbance, $a$ is the absorption coefficient, $b$ is the effective path length, $c$ is the sample concentration, $I_0$ is the radiation emitted by the spectrometer, and $I$ is the transmitted radiation of the sample [84]. All IR spectra were recorded with the ReactIR 3.0 data acquisition software and each spectra consisted of 128 scans at a resolution of $4\text{ cm}^{-1}$. To determine the concentration of L-glutamic acid in water, several solutions of known concentration have been prepared at $45^\circ\text{C}$ and the height of the carboxylate stretching band at $1408\text{ cm}^{-1}$ from the baseline at $1380\text{ cm}^{-1}$ in the IR spectra has been used to obtain the calibration as shown in Fig. 6.4.
Figure 6.4: Calibration of the ATR-FTIR absorbance of the carboxylate stretching band at 1408 cm⁻¹ against known L-glutamic acid concentrations in water at 45°C.

**Solubility measurement of α and β polymorphs**

The solubility of both polymorphs in water has been determined in a range from 20 to 60°C in slurry experiments using ATR-FTIR spectroscopy. Since IR spectra are temperature dependent, calibrations as described in the previous section have been performed at different temperatures. To determine the solubility of both polymorphs, crystals of the corresponding polymorph have been added in excess to an undersaturated solution, and the liquid phase concentration has been measured until no further concentration change was observed. This procedure was repeated at the temperatures where the system was calibrated before. Fig. 6.5 shows the measured aqueous solubilities of the two polymorphs, together with solubility data found in the literature [176]. The experimental error was found to be below 1.5% for all measurements and the measured solubilities of α and β agree well with the literature values. It
Figure 6.5: Solubility of α and β L-glutamic acid in water as a function of temperature. • and ◆ represent the solubility of α and β measured by ATR-FTIR spectroscopy in this work, respectively. The lines are guides to the eye. For comparison, the gravimetrically determined solubility data of α and β L-glutamic acid reported by Sakata [176] is shown as o and ◆, respectively.

It is worth noting, that above a temperature of 50°C it was not possible to obtain reliable solubility values for the metastable α form since the transformation to the stable β was faster than the attainment of equilibrium. The solubility of α is higher than the one of β over the whole temperature range, thus indicating that the system is monotropic. At 45°C, the solubility of the α and β form in water is 21.7 and 17 g/kg solvent, respectively; these values will be used in the model equations.
6.2 Experimental

Figure 6.6: Raman powder spectra detail of the metastable $\alpha$ and the stable $\beta$ form of L-glutamic acid.

6.2.4 Polymorph characterization using in situ Raman spectroscopy

Several analytical methods have been used to characterize and quantify polymorphic crystalline material offline, i.e. X-ray diffraction, solid-state NMR, vibrational spectroscopy, and thermal analysis [177]. However, for this purpose only two techniques have been applied so far in situ during crystallization: X-ray diffraction and Raman spectroscopy [133, 178]. In this chapter we used a RA 400 Raman spectrometer (Mettler-Toledo, Greifensee, Switzerland), equipped with a 250 mW frequency stabilized laser diode at 785 nm and a thermoelectrically cooled Raman detector. The spectrometer is connected via a fiber optic to a 5/8” ball type immersion Reaction RamanProbe from Inphotonics (Norwood, USA) with a wetted length of 330 mm that allows for spectra acquisition within a spectral range from 200 to 3900 cm$^{-1}$ Stokes at a resolution of 3.6 cm$^{-1}$. 
To record spectra with a satisfying peak-to-noise ratio the sample exposure time was set to 45 s and 10 scans were averaged to give one Raman spectra. Raman powder spectra of pure α and β L-glutamic acid crystals are shown in Fig. 6.6, where similarities and differences can be seen.

**Quantification of polymorphic content**

Methods for the quantification of the polymorphic content during crystallization have been proposed in the literature [133, 134, 138]. All methods are based on ex-situ calibration using prepared polymorph mixtures, either in suspension [133, 138] or as dry powder [134]. No influence of the crystal size on the calibrated signal has been noted or reported. In this chapter, we have tried to calibrate the polymorphic content of L-glutamic acid in a similar way. In a first step, a series of calibration samples with different mass ratios of solid α and β particles were suspended in a saturated solution of L-glutamic acid at 45°C. Then, the polymorphic composition was calculated using the characteristic peaks at 1004 cm\(^{-1}\) for α and at 941 cm\(^{-1}\) for β by employing the following equation:

\[
x_\alpha = f \left( \frac{A_\alpha}{A_\alpha + A_\beta} \right)
\]  

(6.2)

where \(x_\alpha\) is the mass fraction of suspended α crystals, \(A_\alpha\) and \(A_\beta\) are the baseline integrated area of the corresponding peaks in the Raman spectrum. Since not only the polymorphic concentration but also the crystal size of the suspended crystals changes during the course of the transformation process, we have repeated the calibration with the same β fraction but a second population constituted of coarser α crystals. Fig. 6.7 shows the results obtained for the two calibration sets, as well as the volume-weighted chord length distribution of the α populations measured by FBRM. Although the trends are similar, the differences between the two calibrations are as large as 10 wt.%. These results indicate that the measured Raman signal is a function not only of the polymorphic content, but also of the crystal size and of the crystal size distribution. Thus, the Raman data presented throughout this chapter was not calibrated but scaled to fulfill the material balance as discussed in section 6.3.1.
6.2 Experimental

Figure 6.7: Experimental results for the calibration of two different α populations in suspension with β crystals. The volume-weighted CLD of both α populations is shown in the upper left corner. The population of β crystals was the same for both calibrations.

6.2.5 Monitoring the particle size using FBRM

The Focused Beam Reflectance Method (FBRM) allows for in situ measurements of the chord length distribution (CLD) of the particle population even at high solid concentrations. It has been used for various purposes in the field of crystallization, such as the measurement of the solubility and of the metastable zone width [117], the estimation of crystallization kinetics [119], or the control of fines [128]. The principle of the measurement technique is described in detail elsewhere [148]. In all experiments we have used a laboratory scale FBRM 600L from Lasentec (Redmond, USA).
6.2.6 Optical image acquisition

In-situ high-resolution images have been taken throughout the processes using a Lasentec Particle Vision and Measurement (PVM) 800 probe (Redmond, USA). Six independent laser sources inside the PVM probe illuminate a fixed area at the probe tip. The backscattered light is focused on a CCD camera producing an image of $1760 \mu m \times 1320 \mu m$ with a resolution of approximately $10 \mu m$. The images yield time-resolved qualitative information about the average particle size and polymorph content since the two polymorphs of L-glutamic acid exhibit different shapes (see Fig. 6.2).

6.2.7 Unseeded experiments

At the beginning of all unseeded experiments, L-glutamic acid was dissolved in deionized water at $80^\circ C$ to yield the desired initial concentration $c_0$. After complete dissolution of the solid material, the solution was cooled at a rate of $1.5 K/min$ to a temperature of $45^\circ C$, which was then held constant throughout the process. Upon reaching $45^\circ C$ the data acquisition of all in situ monitoring tools was started. It is worth noting, that no nucleation could be observed during the cooling phase, hence the crystallization process that follows can be considered to be isothermal.

6.2.8 Seeded experiments

Seeded experiments have been performed by preparing a saturated solution with respect to the $\alpha$ polymorph at $45^\circ C$, i.e. at a concentration of $21.7 g/kg$ solvent. A sufficient amount of $\alpha$ crystals has been produced as described in section 6.2. Two different seed populations have been prepared: a fine population of seed crystals obtained by milling $\alpha$ crystals that passed through a $250 \mu m$ sieve; a coarser population consisting of the sieve fraction between 250 and $500 \mu m$. The mass of seed crystals was chosen to be $26.3 g/kg$ solvent, thus corresponding to the mass of $\alpha$ crystals produced in the first phase of an unseeded experiment with an
initial concentration of 48 g/kg solvent. Upon addition of the α seed crystals, the data acquisition of all in situ monitoring tools was started. It is worth noting, that no nucleation or growth of α crystals could occur during the seeded experiments since the added α seeds were suspended in a saturated solution. Yet, the suspension was supersaturated with respect to the stable β form that could therefore nucleate and grow, thereby consuming supersaturation and eventually triggering the dissolution of the α crystals.

6.3 Experimental results

In this section, we report two series of experiments, i.e. unseeded (section 6.3.2) and seeded experiments (section 6.3.3). Before that, in section 6.3.1 a single reference case is taken as example of the whole set of experiments, in order to describe and discuss in detail the results obtained and the information we can extract from the data collected from the different instruments used to monitor the process.

6.3.1 Unseeded experiments: reference case

Let us consider the unseeded experiment carried out with an initial concentration of 43 g/kg solvent. Information obtained during the course of this experiment (20 hours) from ATR-FTIR, PVM, Raman, and FBRM are reported in Figures 6.8, 6.9, 6.10, and 6.11, respectively. Fig. 6.8 shows the time evolution of the L-glutamic acid concentration measured from ATR-FTIR spectra using the calibration illustrated in Fig. 6.4 during the formation of α crystals and their following transformation into β crystals. These phenomena are clearly recognized when considering the PVM images in Fig. 6.9, where the prismatic α crystals present at the beginning of the experiment are replaced by β needles while the experiment proceeds. Also shown in Fig. 6.8 is the IR absorbance at 1120 cm$^{-1}$ (uncalibrated signal shown in arbitrary units) which indicates the presence of solid particles on the ATR-FTIR probe window. It can be readily observed that whenever the latter signal indicates presence of particles
6. L-glutamic acid polymorphic transformation

Figure 6.8: Solute concentration profile during the transformation process at 45°C with an initial concentration of 43 g L-glutamic acid / kg solvent. Below the concentration profile the scaled absorbance data of the band at 1120 cm\(^{-1}\) is shown to indicate probe clogging during the beginning of the process. Independently measured solubility of \(\alpha\) and \(\beta\) polymorphs are shown by horizontal solid and dashed lines, respectively.

also the main signal used to calculate concentration loses accuracy. The measurement could be continued only after mechanical cleaning of the window; this was done five times during the first five hours of this experimental run. It is worth noting, that ATR-FTIR was the only in situ technique where probe clogging had significant effects on the recorded data. Although the ATR probe tip was in a reactor region with high liquid velocities, particle formation on the ATR crystal during the early stages of the experiment could not be completely prevented. This is probably due to the recessed position of the ATR crystal at the probe tip, which is more prone to solid formation than the planar probes used by the other instruments. With reference to the concentration profile in Fig. 6.8 it is worth noting that apart from the small bumps before probe cleaning, its regularity and accuracy are satisfactory.
Figure 6.9: In-situ PVM images of the solvent mediated transformation process at 45°C with an initial concentration of 43 g L-glutamic acid / kg solvent: after one hour the prismatic α crystals prevail, after six hours both polymorphs are present, and after 16 hours only needle-like β crystals are visible.
Figure 6.10: Profiles of α and β polymorph concentrations during the transformation process with an initial concentration of 43 g L-glutamic acid / kg solvent at 45°C. The profiles have been calculated using the baseline integrated area of the peaks at 1004 cm\(^{-1}\) and 941 cm\(^{-1}\) for α and β form, respectively. Since the Raman data was not calibrated, the profiles were scaled to fulfill the mass balance for easier comparability.

Fig. 6.10 shows the Raman profile of the normalized peak area at 1004 cm\(^{-1}\), which is associated to the α form, and that of the peak at 941 cm\(^{-1}\) which is associated to the β form. Despite the measured signal exhibit significant scattering, the evolution of the two polymorph concentrations can be clearly followed. Thus, the comparison of ATR-FTIR (Fig. 6.8) and Raman spectroscopy (Fig. 6.10) data allows for the identification of the fundamental mechanisms that govern the transformation process. Three different phases can be identified. First, the solute concentration drops rapidly from the initial value to a concentration plateau corresponding to the solubility of the α form, whereas the Raman signal characteristic of the α form rises and reaches its maximum and that of
the β form remains close to zero. This corresponds to the nucleation and growth of the metastable α polymorph. During the second phase, a duration of about six hours in this case, the solute concentration remains constant while the intensity of the Raman α signal decreases and that of the β signal increases. This indicates the occurrence of the solid phase transformation, where the α form dissolves and the β form nucleates and grows. As long as α crystals are present, the solute concentration remains at the solubility level of α. Therefore we can conclude, that not the dissolution of α but the nucleation and growth of β are the rate controlling step during the transformation process. The third phase starts with the drop of the solute concentration and of the Raman α signal that reaches zero. Both effects are due to the dissolution of the last α crystals. During this phase, the Raman β signal reaches in turn its final level, together with the solute concentration profile, which stabilizes at the solubility of the β form. This is the end of the transformation process. Based on these observations, and for the sake of readability, all Raman signals in this chapter have been rescaled to fulfill the overall material balance at least at two points in time during the transformation process. These are when the α form maximum occurs and the solute concentration is at the solubility of α form, and at the final state where the solute concentration coincides with the solubility of the β form, whose Raman signal has reached its final plateau value.

The evolution of the CLD monitored by FBRM during the same transformation process is evaluated by considering the counts in three classes, i.e. 1 to 10, 10 to 100, and 100 to 1000 μm, as shown in Fig. 6.11. The CLD data are rather difficult to interpret quantitatively, particularly when particles with so different shape like α and β crystals are present. However, changes in the FBRM counts are always due to the occurrence of changes in the particle population. This is evident in Fig. 6.11 where the FBRM signal indicates a very rapid event occurring at the beginning of the experiment, i.e. the nucleation of the α crystals and a slower event that takes place between about 1 hour and 7.5 hours during the course of the experiment, i.e. the solid phase transformation. The spikes during the first five hours are probably associated to the disturbances due to the cleaning of the ATR-FTIR probe window.

The combination of the different analytical techniques used helps to elucidate the fundamental mechanisms involved as well as to quantify
Figure 6.11: Profile of the FBRM chord length distribution during the transformation process with an initial concentration of 43 g L-glutamic acid / kg solvent at 45°C. The CLD is merged into three classes of 1-10, 10-100 and 100-1000 μm. The nucleation of α crystals can be observed by a steep increase in the fines count at the beginning of the process.

their effect. ATR-FTIR spectroscopy yields the accurate time evolution of the transformation process by monitoring the solute concentration. Raman spectroscopy is able to provide direct information about the polymorphs present, and their relative amount, at least qualitatively. It can be particularly effective when polymorphs cannot be distinguished by their shape. The CLD profile measured by FBRM contains valuable information about nucleation events and the evolution of the average particle size. Finally, thanks to the use of PVM the process can also be visually followed.
6.3.2 Unseeded experiments: Effect of initial supersaturation

Let us consider first a series of three unseeded experiments, in which we have studied the effect of initial supersaturation on the transformation process by varying the initial solute concentration, $c_0$ (see Table 6.1). The supersaturation $S_i$ with respect to the two polymorphs is defined as the ratio between the actual solute concentration, $c$, and the solubility of the corresponding polymorph, $c_i^*$:

$$S_i = \frac{c}{c_i^*}; \quad (i = \alpha, \beta) \quad (6.3)$$

Online ATR-FTIR and Raman data have been used to determine the solute and polymorph concentration profiles shown in Figs. 6.12 and 6.13. From the FBRM log data the CLD of the population of $\alpha$ crystals after nucleation (1 h after the start of the experiment) and of the final $\beta$ needles have been obtained (see Fig. 6.14).

<table>
<thead>
<tr>
<th>$c_0$ [g/kg solvent]</th>
<th>$S_\alpha$ [-]</th>
<th>$S_\beta$ [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>48</td>
<td>2.2</td>
<td>2.8</td>
</tr>
<tr>
<td>53</td>
<td>2.4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 6.1: Unseeded experiments: Initial concentration and corresponding initial supersaturation with respect to the $\alpha$ and $\beta$ polymorphs at 45°C for the experiments discussed in section 6.3.2.

As expected, in Fig. 6.12 the concentration values of the intermediate and final plateaus are the same in all three experiments, and equal to the solubilities of the $\alpha$ and of the $\beta$ forms, respectively. As shown in Fig. 6.13, and even more precisely in Fig. 6.12 itself, the transformation from the $\alpha$ to the $\beta$ polymorph takes place at the same time in all three cases. The CLDs shown in Fig. 6.14 indicate very similar average particle sizes for the three experiments. As a consequence, the number of crystals has to increase with increasing concentration in order to obey the mass balance; this is evident in the rising number of FBRM counts.
Figure 6.12: Experimental and simulated solute concentration profiles during the transformation process with three different initial concentrations of 43, 48, and 53 g L-glutamic acid / kg solvent, respectively. The calculated profiles and the experimental data overlap and are not always distinguishable.

with increasing initial concentration. The fact that there are more FBRM counts associated to the $\beta$-CLDs than to the $\alpha$-CLDs is not surprising
for two reasons. First, the aspect ratio of the two polymorphs is very...
different \((k_{\alpha}=0.52, k_{\beta}=0.01)\), thus in the case of similar size leading to a larger number of \(\beta\) crystals. Secondly, the mass of \(\beta\) crystals at the end of the transformation is larger due to the lower solubility of the \(\beta\) form. However, it is not obvious why the average sizes of the \(\alpha\) crystals in the three experiments are similar, and so are those of the \(\beta\) crystals in the three experiments. The nucleation and growth kinetics of \(\alpha\) depend on the supersaturation \(S_\alpha\), and should therefore change when changing the initial concentration (see Table 6.1). For the \(\beta\) crystals, one could expect similar final CLDs, since the supersaturation \(S_\beta\) remains constant at the value \(S_\beta = c_\alpha^*/c_\beta^*\) during the second phase of the process in all experiments hence primary nucleation and growth kinetics of \(\beta\) do not change. However, the surface of the \(\alpha\) crystals present at the beginning of the transformation process (end of phase 1) increases with the number of \(\alpha\) crystals and has therefore an influence on the secondary nucleation of \(\beta\) crystals. These two counteracting mechanisms lead to the observed behavior, i.e. to the evidence that the change of initial supersaturation has no significant influence on the transformation times of the process in the concentration range studied. Therefore, when modelling such process the governing kinetic equations for nucleation and growth of \(\alpha\) and \(\beta\) crystals must reflect the fact that the time evolution of the transformation process is the same independent of the initial supersaturation, i.e. a feature of the system that could not be anticipated.

### 6.3.3 Seeded experiments

Two seeded experiments, labelled Exp 1 and Exp 2, have been performed using seed populations with different average particle size, but the same mass as described in section 6.2.8. Fig. 6.15 shows the CLDs of the two seed populations measured before the experiment, and the CLDs of the obtained crystals. Figs. 6.16 and 6.17 show the solute and solid concentration profile, similarly to Figs. 6.12 and 6.13 for the unseeded experiment. Seeded experiments like these are useful to decouple the crystallization kinetics of the two polymorphs, since nucleation and growth in this case can occur for the stable polymorph only, whereas the metastable polymorph can only dissolve. On the contrary, during the unseeded experiments described earlier phenomena involving both polymorphs occur
Figure 6.14: Experimental square weighted chord length distribution of the solvent mediated transformation process at 45 °C with three different initial concentrations of 43, 48, and 53 g L-glutamic acid per kg solvent, respectively. The CLD on the left side have been recorded one hour after the start of the process and show the distribution of α crystals. The CLD on the right side have been recorded after 16 hours at the end of the transformation process, and show the final CLD of the β crystals.

simultaneously, hence it is more difficult to evaluate the influence of process parameters on each of them separately.

With reference to Fig. 6.15 it can be observed that, having identical mass the seed population of Exp 1 with a smaller average particle size has a larger number of crystals than the coarser seed crystals of Exp 2. It is worth noting, that the final CLDs of the two populations are slightly different, i.e. the final CLD of Exp 1 exhibits a smaller average particle size than the final CLD of Exp 2; this is contrary to the case of the final CLDs measured after the unseeded experiments with different initial supersaturation (see Fig. 6.14). Fig. 6.16 shows the corresponding solute
Figure 6.15: Initial and final square weighted chord length distributions of the two seed populations for the seeded transformation experiments at 45°C. ▲ and ■ represent the initial and final CLD of the population with a smaller average particle size used in Exp 1, whereas △ and □ denote the initial and final CLD of the population used in Exp 2, respectively.

With respect to Fig. 6.16, one can notice that the signal during the initial phase of both experiments are disturbed by probe clogging phenomena. Nevertheless, the evolution of the solute concentration can be clearly followed in both cases. As expected, the solute concentration evolves from the initial plateau corresponding to the solubility of the α form, to the final plateau corresponding to the solubility of the β form. Accordingly, the solid concentration profiles in Fig. 6.17 show the transformation from the α to the β form. However, in the case of the seeded experiments the solid transformation process is completed much earlier in the case of Exp 1 than in Exp 2. This is a noticeable difference with respect to the
Figure 6.16: Experimental solute concentration profiles during the seeded transformation process with two different seed populations at 45°C. • and ○ represent the solute concentration obtained by seeding with the seed populations used in Exp 1 and 2 shown in Fig. 6.15, respectively.

The larger transformation velocity observed in Exp 1 than in Exp 2 must be related with the different features of the two seed population since all other process parameters, i.e. initial supersaturation, seed mass, and temperature, were equal in the two experiments. The key difference between the two seed populations is average size, hence the different number of crystals present, which results in a significant difference in the seed crystal surface at the beginning of the transformation process. With respect to the mechanisms of nucleation and growth of the stable β form, such a difference in available crystal surface can only have an impact on the secondary nucleation kinetics of β crystals, while the growth kinetics is unaffected. In order to check that this indeed takes place, we have devised an experiment to follow the secondary nucleation of β on the
Figure 6.17: Experimental profiles of α and β polymorphs during the seeded transformation process at 45°C. The profiles have been calculated using the baseline integrated area of the peaks at 1004 cm\(^{-1}\) and 941 cm\(^{-1}\) for α and β form, respectively. Since the Raman data was not calibrated, the profiles were scaled to fulfill the mass balance for easier comparability.

Surface of α crystals. An L-glutamic acid solution saturated with respect to the α form was prepared at 45°C. Several large α crystals were added to the solution, whose evolution was followed using PVM. The solution was not stirred during the experiment to facilitate the observation. As shown in Fig. 6.18, we could clearly observe β needles forming on the surface of α crystals and growing into the surrounding solution. Such observation is in agreement with recent literature data [179, 180].
6.4 Mathematical model of the polymorph transformation

Figure 6.18: In-situ PVM image of the surface nucleation of the stable $\beta$ form on the surface of a metastable $\alpha$ crystal. The image has been recorded in a stagnant saturated solution with respect to $\alpha$ at 45°C, where $\alpha$ seed crystals were initially added. A series of PVM images documented the nucleation and subsequent growth of $\beta$ in the surface of $\alpha$. Two white circles indicate the growing $\beta$ crystals in the upper image.

6.4 Mathematical model of L-glutamic acid crystallization and solid phase transformation

In this section, a mathematical model of the crystallization of L-glutamic acid and of the solvent mediated transformation of the metastable $\alpha$ form into the stable $\beta$ form is presented. This is based on population balance equations (PBE). The model is first used to estimate the parameters appearing in the functional relationships for kinetics of nucleation and growth from the experiments presented above. Then, once validated, the model allows a deeper and broader analysis of the process under examination.
6.4.1 Model equations

A mathematical model based on population balance equations has been developed in order to describe the crystallization process of L-glutamic acid from its supersaturated solution followed by its solvent mediated polymorphic transformation. The model describes the time dependent particle size distributions \( n_i(t, L) \) of each polymorph which obeys the following population balance equations [161]:

\[
\frac{\partial n_i}{\partial t} + G_i \frac{\partial n_i}{\partial L} = 0; \quad (i = \alpha, \beta)
\]

where \( L \) is the crystals size \( G_i \) is the mean crystal growth rate of the i-th polymorphs. The surface-integration controlled, size-independent growth of the two polymorphs is described by two exponential equations proposed by Kitamura, that characterize the growth of L-glutamic acid polymorphs through the nuclei-above-nuclei (NAN) model [53]. The molar concentration \( c \) of the solute in the liquid phase fulfills the following equation:

\[
\frac{dc}{dt} = -\frac{1}{2M} \left[ k_{\alpha\alpha} \rho \alpha G_{\alpha} \int_0^\infty n_{\alpha} L^2 dL + k_{\alpha\beta} \rho \beta G_{\beta} \int_0^\infty n_{\beta} L^2 dL \right], \quad (6.5)
\]

where \( M \) is the molar mass of the solute, \( \rho_i \) and \( k_{ai} \) are the solid density and the surface shape factor of the i-th polymorph, respectively. For the sake of simplicity we have used the value of 1540 kg/m\(^3\) for the solid density of both polymorphs. The following initial and boundary conditions apply:

\[
c(0) = c_0, \quad (6.6)
\]

\[
n_i(0, L) = 0; \quad (i = \alpha, \beta) \quad (6.7)
\]

\[
n_i(t, 0) = \frac{J_i}{G_i}; \quad (i = \alpha, \beta) \quad (6.8)
\]

with \( c_0 \) being the initial concentration of the solute and \( J_i \) the nucleation rate per unit volume of the i-th polymorph. The nucleation rate equations used in this model account for the primary heterogeneous nucleation of the \( \alpha \) and \( \beta \) forms, since it is reasonable to neglect homogeneous nucleation under the experimental conditions described in this
### Mathematical model of the polymorph transformation

<table>
<thead>
<tr>
<th>Fundamental mechanism</th>
<th>Kinetic expression</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>α heterogeneous nucleation</td>
<td>$J_\alpha = k_n \alpha S_\alpha^{7/3} \exp \left( \frac{-K_n \alpha}{\ln S_\alpha} \right)$</td>
<td>$k_n, K_n$</td>
</tr>
<tr>
<td>α size independent growth</td>
<td>$G_\alpha = k_g \alpha (S_\alpha - 1)^{5/6} \exp \left( \frac{-K_g \alpha}{S_\alpha - 1} \right)$</td>
<td>$k_g, K_g$</td>
</tr>
<tr>
<td>α dissolution</td>
<td>$D_\alpha = k_{d\alpha} (1 - S_\alpha)$</td>
<td>$-$</td>
</tr>
<tr>
<td>β heterogeneous and surface nucleation</td>
<td>$J_\beta = k_n \beta S_\beta^{7/3} \exp \left( \frac{-K_n \beta}{\ln S_\beta} \right)$</td>
<td>$k_n \beta, K_n \beta$</td>
</tr>
<tr>
<td>β size independent growth</td>
<td>$G_\beta = k_g \beta (S_\beta - 1)^{5/6} \exp \left( \frac{-K_g \beta}{S_\beta - 1} \right)$</td>
<td>$k_g \beta, K_g \beta$</td>
</tr>
</tbody>
</table>

Table 6.2: Fundamental mechanisms, their kinetic expressions, and the according parameters describing the crystallization kinetics of both polymorphs in the population balance model, with $k_n$: pre-exponential factor nucleation rate [\# m$^{-3}$ s$^{-1}$]; $K_n$: exponential factor nucleation rate [-]; $k_g$: pre-exponential factor growth rate [m s$^{-1}$]; $K_g$: exponential factor growth rate [-]; $k_{d\alpha}$: α dissolution rate constant [m s$^{-1}$]; $k_{s\beta}$: pre-exponential factor β surface nucleation rate [\# m$^{-2}$ s$^{-1}$]; $m_2^\alpha = \int_0^\infty L^2 n dL$: second moment of the α particle size distribution [m$^2$ m$^{-3}$]; $K_{s\beta}$: exponential factor β surface nucleation rate [-].

Furthermore, the surface nucleation of β on the surface of α crystals has been included since this phenomenon has been observed experimentally as discussed in the previous section. The correlations used for the nuclelation rate are those of the classical nucleation theory [6]. It is worth noting, that the dissolution of the metastable α form has been considered to be mass transfer limited with the mass transfer coefficient $k_{d\alpha}$ has been predicted using the Sherwood correlation [6]:

$$k_{d\alpha} = \frac{D}{L} \left( 2 + 0.8 \left( \frac{\bar{e} L^4}{\nu^3} \right)^{1/5} Sc^{1/3} \right)$$

(6.9)

where $D$ is the diffusivity, $L$ the crystal size, $\bar{e}$ the average power input, $\nu$ the kinematic viscosity and $Sc$ the Schmidt number, i.e. $Sc = \nu/D$. All fundamental phenomena included in the population balance model are reported in Table 6.2, together with the corresponding kinetic equations and parameters.

The population balance equations are solved using PARSIVAL©, a
commercial solver for integro-differential equations [181]; the program employs a Galerkin \( h, p \)-method based on a generalized finite-element scheme with a self-adaptive grid.

### 6.4.2 Parameter estimation

The equations describing the nucleation and growth kinetics of \( \alpha \) and \( \beta \) L-glutamic acid contain ten parameters that have been estimated using the experimental data obtained during the unseeded transformation processes at different initial concentrations. In particular, the characteristic evolution of the solute concentration recorded by ATR-FTIR spectroscopy, the relative polymorphic content followed by Raman spectroscopy, the average particle size, and the final particle size as measured by PVM and FBRM were used. The set of parameters yielding the best fit is reported in Table 6.3. The calculated solute and solid phase concentration profiles during the three unseeded experiments are compared to the experimental results in Figs. 6.12 and 6.13, respectively. The simulated solute concentration profile agrees very well with the experimental data, whereas the scattering of the Raman experimental data makes their agreement with simulation results less satisfactory but still more than acceptable.

### 6.4.3 Process analysis

The mathematical model described above was used together with the estimated kinetic parameters to analyze two different process parameters of the seeded solid phase transformation, i.e. the particle size distribution of the seed fraction and the suspension density. As experimentally observed, a smaller average particle size of the seed fraction should lead to higher transformation velocities, whereas a change of the suspension densities, which was induced by the difference in initial supersaturation in the unseeded experiments, should have no effect on the transformation. First, the effect of average particle size of the seed fraction was studied using two different seed populations as shown in Fig. 6.19. Similar to the experimental results presented in Figs. 6.16 and 6.17, the
Table 6.3: Estimated set of parameters describing the crystallization kinetics of both polymorphs during the solvent-mediated polymorphic transformation at 45°C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{n\alpha}$ [m$^{-3}$ s$^{-1}$]</td>
<td>$8.0 \times 10^{+5}$</td>
</tr>
<tr>
<td>$K_{n\alpha}$ [-]</td>
<td>$1.0 \times 10^{-1}$</td>
</tr>
<tr>
<td>$k_{g\alpha}$ [m s$^{-1}$]</td>
<td>$2.5 \times 10^{-7}$</td>
</tr>
<tr>
<td>$K_{g\alpha}$ [-]</td>
<td>$9.0 \times 10^{-2}$</td>
</tr>
<tr>
<td>$k_{v\alpha}$ [-]</td>
<td>$5.2 \times 10^{-1}$</td>
</tr>
<tr>
<td>$k_{n\beta}$ [m$^{-3}$s$^{-1}$]</td>
<td>$5.4 \times 10^{+4}$</td>
</tr>
<tr>
<td>$K_{n\beta}$ [-]</td>
<td>$1.5 \times 10^{+1}$</td>
</tr>
<tr>
<td>$k_{s\beta}$ [m$^{-2}$ s$^{-1}$]</td>
<td>$6.0 \times 10^{+4}$</td>
</tr>
<tr>
<td>$K_{s\beta}$ [-]</td>
<td>$1.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>$k_{g\beta}$ [m s$^{-1}$]</td>
<td>$6.5 \times 10^{-8}$</td>
</tr>
<tr>
<td>$K_{g\beta}$ [-]</td>
<td>$1.6 \times 10^{-1}$</td>
</tr>
<tr>
<td>$k_{v\beta}$ [-]</td>
<td>$1.0 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

solute and solid concentrations shown in Figs. 6.20 and 6.21 exhibit a faster transformation of the process seeded with the population with a smaller average particle size. Second, we analyzed the influence of the suspension density during a seeded transformation process. Therefore, the seed population with a smaller average particle size from the previous study (shown as Run 1 in Fig. 6.19) was used with three different seed masses of 21.3, 26.3, and 31.3 g per kg solvent. These values correspond to the maximum amount of α crystals during the second phase of the unseeded transformation process presented in section 6.3.2. Figs. 6.22 and 6.23 show the calculated profiles of the solute and solid concentrations of α and β for the three seed masses, respectively. It can be readily observed, that a change of the seed crystal mass, which corresponds to a change of initial concentration of the unseeded experiments, has no significant influence on the course of the transformation process in the supersaturation range studied. Since the particle size distributions used during this process analysis have been chosen arbitrarily, the re-
Figure 6.19: Mass density particle size distributions of two seed populations used in the simulation study. Both populations differ in their average particle size and distribution width, but have the same total mass.

results can not be directly compared to the transformation experiments. Yet, the computed results agree very well with the experimental findings in a qualitative way and therefore allow for a better understanding of the phenomena appearing during the transformation process. On the one hand it was shown, that seed fractions with smaller average particle size lead to higher transformation velocities during seeded experiments. The reason for the increased transformation velocity must be related with the nucleation kinetics of the $\beta$ form, since the $\beta$ growth kinetics are not varied during the transformation experiments due to the constant supersaturation level which is defined by the difference in solubility of the two polymorphs. This suggests that the available seed crystal surface, which governs the expression of $\beta$ surface nucleation, must have a significant influence on the course of the process. On the other hand we have ob-
served that varying the suspension density did not have an effect on the transformation velocity within the studied range. This brings us to the conclusion, that the two parts of the expression describing the nucleation rate of $\beta$ that are shown in Table 6.2 have a highly different impact. The first part of the expression describing the heterogeneous nucleation of $\beta$ has no significant influence on the course of the process, since the change of initial supersaturation in the unseeded experiments has no effect on the transformation velocities. On the contrary, the second part of the expression describing the surface nucleation on the metastable $\alpha$ form has the major impact on the transformation velocity due to its dependence on the available $\alpha$ surface. Consequently, the overall transformation velocity depends mainly on the available surface of $\alpha$ crystals. This conclusion is supported by the research of Davey and coworkers [28], who have reported the stabilization of the metastable $\alpha$ form of L-glutamic acid by conformational mimicry, i.e. the adsorption of a suitable additive on the
Figure 6.21: Simulated solid concentration profiles of $\alpha$ and $\beta$ during the seeded transformation process with the populations featured in Fig. 6.19.

$\alpha$ crystal surface to prevent surface nucleation of the stable $\beta$ form.

6.5 Conclusions

Four different in situ analytical instruments, namely ATR-FTIR spectroscopy, Raman spectroscopy, FBRM, and PVM have been combined to monitor the solvent-mediated polymorphic transformation of L-glutamic acid during unseeded and seeded processes. All information acquired with the in situ tools helped to identify the fundamental phenomena that govern the transformation process. The characteristic process information extracted from the analytical data has been used to estimate the parameters of the first order equations that describe the nucleation and growth kinetics of both polymorphs in the population balance model. Finally, a
Figure 6.22: Simulated solute concentration profiles during the seeded transformation with three different seed masses of 21.3, 26.3, and 31.3 g α L-glutamic acid / kg solvent which are represented by a dotted, dashed, and solid line, respectively.

simulation study allowed a deeper understanding of the influence of two process parameters, i.e. the suspension density and the surface of a seed population. The combination of in situ analytical tools and mathematical modelling will allow for faster and more robust process development of polymorphic systems in the future, particularly in the pharmaceutical industry. The methods proposed in this article will be used further to study other polymorphic systems to check their applicability on a larger scale.
Figure 6.23: Simulated solid concentration profiles of $\alpha$ and $\beta$ during the seeded transformation with three different seed masses of 21.3, 26.3, and 31.3 g $\alpha$ L-glutamic acid / kg solvent which are represented by a dotted, dashed, and solid line, respectively.
Chapter 7

Antisolvent precipitation of PDI 747: kinetics of particle formation and growth

The application of two previously developed protocols for the fast and robust determination of nucleation and growth kinetics to the antisolvent precipitation of PDI 747 is presented in this chapter. Both characterization methods are based on two in situ process analytical technologies, i.e., ATR-FTIR spectroscopy and FBRM to monitor the liquid and the solid phase during the characterization experiments. The method to determine growth rate kinetics is based on seeded batch desupersaturation experiments. It combines the accurate characterization of seed particle size distributions and the measurement of desupersaturation profiles for different experimental conditions with population balance modeling and an optimization routine to determine the growth mechanism and the kinetics parameters. In a second step, the growth kinetics are combined with induction time experiments to determine the nucleation rate. Finally, the comparison of simulation and experimental results indicates the importance of additional phenomena, such as particle breakage and agglomeration.
7.1 Introduction

Precipitation processes from solution are used in the pharmaceutical and fine chemical industry to manufacture particulate products in the micron and submicron range. Defined product properties such as dissolution rates, bioavailability or possible drug delivery via inhalation require distinct and consistent crystal product characteristics [182]. Yet, the rigorous design of optimal process conditions to manufacture a particulate product with specific properties requires the knowledge of the kinetics of the governing fundamental mechanisms. Once the particle formation kinetics are known, they can be plugged into a population balance model to simulate the influence of different process parameters on the final particle size distribution (PSD) of the product. Yet, the determination of nucleation and growth kinetics of precipitation processes is up to now not straight forward and can be very time consuming [23].

In this chapter, the application of characterization protocols for determining the growth and nucleation kinetics is presented for the antisolvent precipitation of PDI747. These protocols were recently developed and tested for the precipitation of the metastable α form of L-glutamic acid via pH-shift [49, 183]. Both protocols rely mainly on in situ measurement techniques to characterize the liquid and solid phase during the process, thereby avoiding the need for sampling, which significantly reduces the experimental error and the time required for the kinetics measurement. The application of both nucleation and growth characterization protocols is limited to processes that are slow enough not to be influenced by mixing in a stirred batch reactor. The aim of this chapter is to demonstrate the applicability of the characterization techniques to an industrially relevant system. PDI747 is a phosphodiesterase type 4 inhibitor and was originally developed for inflammatory skin diseases by Novartis [184].

7.2 Materials and methods

Methanol (HPLC Gradient Grade, L.T.Baker, Deventer, The Netherlands) and deionized water were used after filtration over a 0.5 μm fil-
Figure 7.1: Molecular structure of PDI747.

7.2 Materials and methods

PDI747 (C_{26}H_{33}NO_{5}, M = 439.23 kg/kmol, B12 OPK 1/04) was provided by Novartis Pharma AG (Basel, Switzerland) and was used without further purification. The molecular structure of PDI747 is shown in Fig. 7.1. Three polymorphs of PDI747 are reported and known as modifications A, B, and C [185]. Modification C is considered as the thermodynamically stable form at room temperature, whereas polymorph A is the metastable form which transforms upon melting at about 101°C into form B with a melting point of about 118°C [185]. The X-ray powder diffraction pattern of the stable form C is shown in Fig. 7.2. It was recorded with a Bruker (Karlsruhe, Germany) AXS D8 Advance (40kV, 40mA), with 2Θ (Cu-Kα) = 5° - 35°, step = 0.02° and scan speed = 0.5°/min. The sample material was analyzed in both the ground as well as the unground forms to check for possible polymorphic transformations during grinding. None of the two other metastable polymorphs could be produced for characterization purposes despite various recrystallization experiments according to a given experimental protocol [186]. This case of disappearing polymorphism might be related to a different impurity profile of the present raw ma-
Figure 7.2: Experimental X-ray diffraction pattern of the stable polymorph C of PDI747.

Material compared to the sample available when the substance was characterized the first time; similar cases are reported in literature for other substances [187, 188]. Consequently, all solid characterization details and kinetic data reported in this chapter refer to the stable C form. The solid density of PDI crystals was determined to be $\rho_c = 1187 \pm 1.2 \text{kg/m}^3$ using a MVP-1 Multipycnometer$^{\text{TM}}$(Quantachrome, Odelzhausen, Germany). A DSC822$^e$ differential scanning calorimeter from Mettler-Toledo (Greifensee, Switzerland) was used to characterize the melting temperature and melting enthalpy of PDI747, which are 118.1$^\circ$ C and 108 J/g, respectively. In each DSC experiment, about 10 mg of substance were used, the heating rate was set to 10 K/min and the instrument was flushed with pure nitrogen. The onset-temperature in the DSC diagrams was determined as described in the literature and was considered as the melting temperature of the substance [189].
7.2 Materials and methods

7.2.1 Batch crystallizer set-up

The jacketed 500 mL borosilicate glass reactor used throughout all experiments is described in detail elsewhere [49]. The temperature in the crystallizer was controlled with a CC230 thermostat from Huber (Offenburg, Germany) using a PT100 and it was recorded using Labview 6.0 (National Instruments, Austin, Texas, US). Fig. 7.3 shows the experimental setup together with the in-situ measurement instruments used in this chapter. The position of the immersion probes was chosen in the zone of high fluid velocities, i.e., close to the impeller tips, to minimize clogging of the probe windows, thus optimizing the quality of the recorded data.

7.2.2 In situ monitoring techniques

The key advantage of using in situ analytical techniques for process characterization is a good accuracy and repeatability of experimental data since no sample preparation is required [75, 182, 190, 191]. Focused Beam Reflectance Measurement (FBRM) and Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy were used to monitor solid and liquid phase properties during the experiments described in sections 7.2.4 and 7.2.5. FBRM is used to measure in situ chord length distributions (CLDs) of a suspended particle population. The ATR technique enables to record liquid phase IR spectra that are unaffected by the presence of the solid phase due to the small penetration depth of the IR beam into the liquid. The measurement principles of both techniques are described in detail elsewhere [79, 148, 149].

Focused Beam Reflectance Measurement (FBRM)

FBRM was used for two different purposes. First, during seeded growth experiments, the CLDs of the particle populations were monitored to ensure that no significant nucleation occurred over the course of the experiments; second, during induction time experiments, the onset of particle formation was determined based on the total number of particles measured by the FBRM within the size range of 1-11 μm. It is worth noting
that the FBRM was not used as particle sizer to characterize particle size distributions (PSD) since an off line particle analyzing technique using the electrical sensing zone method (Coulter Multisizer 3, Beckman Coulter, Nyon, Switzerland) was considered more accurate and allowed easier comparison of experimental and simulated PSD data. All FBRM measurements in this chapter were carried out using a laboratory scale Lasentec 600L from Mettler-Toledo (Schwerzenbach, Switzerland).
Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectroscopy was used to obtain the solute concentration and the solvent/antisolvent ratio during the experiment. While the knowledge of accurate online solute concentration data was vital mainly for the determination of growth kinetics, the measurement of solvent/antisolvent ratios was of importance for both nucleation and growth rate characterization experiments in order to confirm that the desired experimental conditions were fulfilled in all experiments. The two bands in the IR spectra at 1245 cm\(^{-1}\) and 1654 cm\(^{-1}\) were identified to represent the concentration of the solute PDI747 and the antisolvent water, respectively. These bands were used to build independent univariate calibration models for PDI747 and solvent mixture composition by applying the law of Beer-Lambert [84]. It is worth noting, that such a univariate calibration method can only be accurate when the signal intensity at each of the bands is exclusively influenced by the concentration of the corresponding substance. To overcome that limitation, calibrations for 3-component systems are often based on multivariate approaches such as principal component analysis (PCA) [75, 192]. The calibration for the concentration of PDI747 is given in wt.% of total mass, whereas the solvent/antisolvent composition is calibrated in wt.% on a solute free basis. Pure methanol was always taken as background spectrum. Fig. 7.4 shows that both sets of calibrations exhibit linear behavior with \(R^2\) values close to one in both cases. All ATR-FTIR measurements in this work were performed using a ReactIR 4000 system from Mettler-Toledo (Schwerzenbach, Switzerland), equipped with a 11.75” DiComp immersion probe and a diamond as ATR crystal.

Solubility measurements and supersaturation

The solubility of PDI747 in methanol/water mixtures was determined gravimetrically in a range of methanol mass fractions from 0.2 to 1 at 25°C. First, the solvent/antisolvent mixture was prepared carefully and the solute was added in excess. The slurry was kept in a closed container in a temperature controlled water bath for 48 hours for equilibra-
tion. Then, the saturated solution was filtered, weighed and put into a vacuum oven to separate the liquid from the solid phase at 60°C and 500 mbar. Weighing the remaining dry solute particles yielded together with the weight of the saturated solution the solubility at the given solvent/antisolvent composition. Furthermore, ATR-FTIR spectroscopy was used to validate the solubility values measured gravimetrically in the range of methanol mass fractions above 0.6. For these slurry experiments, PDI747 was added in excess to an undersaturated solution of given solvent/antisolvent composition, and the liquid phase concentration was measured using ATR-FTIR until a stable measurement signal was attained. This procedure was performed three times for each solvent composition to obtain accurate experimental data. The solubility value was obtained by averaging the three measured values. Good agreement with the gravimetrically determined values could be achieved with de-
Figure 7.5: Solubility data of PDI747 in the system water-methanol as a function of solvent composition at 25°C.

Variations below 0.5 % for all measurements. In Fig. 7.5 the solubility of PDI747 is shown as a function of the solvent composition. The supersaturation ratio $S$ that controls the PDI747 particle formation is defined as the ratio of actual concentration $c$ and equilibrium concentration $c^*$ assuming an ideal solution:

$$ S = \frac{c}{c^*(w_{\text{MeOH}})} , $$

where $c$ is calculated using the IR band of the solute at 1245 cm$^{-1}$ and $c^*$ is the solubility value of PDI747, corresponding to the actual solute free methanol mass fraction $w_{\text{MeOH}}$ in the liquid phase (see Fig. 7.5).
7.2.3 Off line particle size analysis

The Multisizer 3 from Beckman Coulter (Nyon, Switzerland) was used for off line particle size analysis. This device employs the electrical sensing zone method which both counts the particles and measures their volume very accurately. The measured volume is used to calculate the diameter of a volume equivalent sphere. The measurement principle of this device and the advantages and drawbacks can be found elsewhere [143]. The electrolyte was prepared by dissolving 1 wt. % NaCl in a mixture of 90 wt. % water and 10 wt. % Ethanol. The solution was filtered several times using a 0.22 μm Millipore filter (Billerica, USA) to minimize the number of counted foreign particles. Each PSD presented in this work is the average of three measured samples and is based on data for at least 210,000 particles. It is worth noting that all experimental PSDs were slightly scattered despite the large number of counted particles. In order to reduce computational time needed for the parameter optimization procedure, all PSDs were smoothed using a filtering algorithm included in the data acquisition of the device which averages the measured data of each size class with the three adjacent lower and upper size classes.

7.2.4 Measurement of growth kinetics

The measurement procedure for the independent determination of growth rate kinetics is based on seeded batch desupersaturation experiments and has been described in detail elsewhere [183]. In brief, a supersaturated solution was created upon mixing a PDI 747 solution and antisolvent in the reactor, the solution consisting of methanol and PDI 747 and the antisolvent being a mixture of water and methanol with a constant 55 to 45 wt.% ratio. The masses of solution and antisolvent were chosen in such a way to obtain a constant methanol mass fraction of 0.6. Then, the in situ monitoring instruments for the liquid and solid phase, i.e. ATR-FTIR and FBRM, were started in order to monitor during the experiment the solute concentration and the solid phase CLDs, respectively. At time zero of the experiment, a certain amount of seeds was fed to the reactor in dry form. All desupersaturation experiments were performed twice. Fig. 7.6 shows the typical repro-
Figure 7.6: Repeatability of two sets of desupersaturation experiments presented in section 7.2.4. ◊, ⋄, □, and ■ represent Run 1, 2, 3, and 4 of the set of experiments given in table 7.1, respectively. The different initial conditions are highlighted in the graph.

ducibility of the measured desupersaturation curves for two repeated runs of experiments at different initial conditions; it can be readily observed that the repeatability was satisfactory in both cases. To allow for the independent determination of growth kinetics the absence of other phenomena, like breakage, agglomeration or nucleation, has to be verified for all experiments. Although it could not be completely prevented due to the elongated characteristic particle shape of PDI 747, crystal breakage was minimized by employing an impeller with rounded edges and by stirring at the minimum speed while assuring complete crystal dispersion in the liquid phase. The absence of significant nucleation was assured by monitoring the CLDs using the FBRM during growth. Typical time resolved CLDs are shown in Fig. 7.7. It can be readily observed, that no change in the counts at small chord lengths
occurred, thus indicating the absence of significant nucleation. Similar results were obtained for all growth characterization experiments. Two experimental parameters, i.e., initial supersaturation and surface of the seed crystals, can be varied in a certain range to assure that the requirements described above are met. While higher supersaturation values generally promote nucleation and agglomeration, a larger seed surface accelerates supersaturation depletion by crystal growth, thus preventing nucleation. Finally, the measured growth rate parameters were estimated by comparison of simulation to experiments at different operating conditions, i.e. initial supersaturation, seed size, and seed mass as described in section 7.4.2.

Figure 7.7: FBRM data of Run 4. It can be easily observed that no significant nucleation occurred over the course of the experiment since the counts of small chords remain at a constant low level.
7.2.5 Determination of nucleation kinetics

Induction time experiments were performed recently to determine the kinetics of primary homogeneous and heterogeneous nucleation of the metastable α form of L-glutamic acid [49]. In this work, we have used the same characterization method which is based on the in situ measurement of the induction time, i.e. the period between the attainment of the desired supersaturation level and the detection of newly formed particles [154], in order to determine the nucleation kinetics of PDI747 during antisolvent precipitation. The proposed method takes full advantage of two monitoring techniques, namely ATR-FTIR and FBRM, to measure the induction time entirely in situ without any sampling procedures. ATR-FTIR monitors the liquid phase to determine the time when the initial supersaturation is established homogeneously throughout the reactor whereas FBRM detects the solid phase which consists of the precipitated nuclei grown to detectable size. All induction time experiments were conducted at 25°C in the batch crystallizer described in section 7.2.1, and were performed by mixing two starting solutions: a PDI solution containing 13.5 wt.% PDI dissolved in methanol and an antisolvent which consisted of a 45:55 wt.% mixture of water and methanol. The latter mixture was chosen to exclude the influence of mixing effects on the measured kinetics since precipitation is extremely fast upon addition of pure water. The initial supersaturation was adjusted in all induction time experiments by varying the relative amount of PDI solution and added antisolvent. Both, PDI and antisolvent solution, were prepared and purified by filtration over a 0.22 μm filter (Millipore, USA) before each experiment. The PDI747 solution was filled in the reactor and the ATR-FTIR and FBRM measurements were started simultaneously. The measurement frequency of both instruments was chosen in such a way that several IR spectra and chord length distributions (CLD) were recorded during the induction period. After measuring initially a few IR spectra and CLDs to confirm the signal stability, the antisolvent was added into the reactor. It is worth noting, that the precise and repeatable measurement of the induction time requires that the time needed to establish the initial supersaturation is short in comparison to the measured induction time in order to exclude any mixing effects. Fig. 7.8 shows the typical evolution of the monitored data during one experiment, i.e. the solute
concentration determined by evaluating the absorbance at 1245 cm\(^{-1}\) in the IR spectrum using the corresponding calibration, and the FBRM counts of the smallest detectable chord lengths, i.e. 1-11 \(\mu\)m. The time profiles in Fig. 7.8 can be divided into four phases. In phase 1, during the initial lag time of about 130 seconds, the signals of both ATR-FTIR and FBRM remain constant. The antisolvent addition in phase 2 is clearly observable in the ATR-FTIR signal, which decreases to the value of the nominal PDI747 concentration, whereas a small bump in the FBRM signal is due to bubble formation. The point in time where the nominal supersaturation conditions have been attained everywhere in the reactor is clearly identified as the point, where the ATR-FTIR signal is again stable and constant. At this point phase 3 begins, which is the induction period. For the determination of the end of the induction period one has to resort to the FBRM counts, which show a distinct increase upon
particle detection as shown in Fig. 7.8. The time period between these two events is the induction time associated to the nucleation and subsequent growth of PDI747 crystals. The fourth phase is characterized by the sharp increase in FBRM counts. Each induction time measurement was repeated at least twice and was performed at different initial supersaturation levels in order to measure the induction time as a function of supersaturation at a temperature of 25°C.

### 7.3 Population balance equations

A mathematical model based on population balance equations (PBEs) is used in combination with a least squares optimization and the experimental desupersaturation data to determine the kinetic parameters of PDI747 growth as presented and discussed in section 7.4. The PBE for size-independent growth, with neither agglomeration nor breakage, is as follows [161]:

\[
\frac{\partial n}{\partial t} + G \frac{\partial n}{\partial L} = 0,
\]

where \(n(t, L)\) is the number density of particles per unit volume of size between \(L\) and \(L + dL\), \(L\) is the characteristic length of the particle, i.e. the length of the needle shaped PDI crystals, and \(G\) is the crystal growth rate of this characteristic length. It is worth noting that particle size of the simulated final PSDs shown in this work have been recomputed from the needle length to the size of a volume equivalent sphere to allow for the direct comparison to the experimental results obtained using the Beckman Multisizer 3. The molar concentration \(c\) of the solute in the liquid phase fulfills the following material balance:

\[
\frac{dc}{dt} = -\frac{3}{M} k_v \rho_c G \int_0^\infty n L^2 dL,
\]

where \(M\) is the molar mass of the solute, \(\rho_c\) and \(k_v\) are the solid density and the volume shape factor of PDI747, respectively. As described in section 7.2, the solid density of PDI747 was determined to be \(\rho_c = 1187 \text{ kg/m}^3\). The volume shape factor was estimated to be about \(k_v = 0.01\) corresponding to an average aspect ratio of 10 in accordance with
the evidence provided by inspection of many different SEM microphotographs. The following initial and boundary conditions apply:

\[ c(0) = c_0 \] \hspace{1cm} (7.4)
\[ n(0, L) = n_0(L) \] \hspace{1cm} (7.5)
\[ n(t, 0) = \frac{J}{G} , \] \hspace{1cm} (7.6)

with \( c_0 \) being the initial concentration of the solute, \( n_0(L) \) the initial PSD and \( J \) the nucleation rate per unit volume. The nucleation rate used in this model accounts for the primary homogeneous and heterogeneous nucleation of PDI 747. For the growth kinetics parameter determination in section 7.4 it was assumed that no nucleation occurred, i.e. \( J = 0 \) in Eq. 7.6. The system of equations above was solved employing a discretization method developed by Kumar and Ramkrishna [162]. It is based on a combination of the discretization along the particle size coordinate \( L \) and of the method of characteristics, which yields improved computational efficiency while overcoming problems of stability and numerical diffusion.

For the estimation of the growth kinetics parameters the following least squares problem had to be solved:

\[
\min \sum_{m=1}^{N_e} R_m^2 = \min \sum_{n=1}^{N_e} \left[ \frac{1}{t_{end}^{(m)}} \int_0^{t_{end}^{(m)}} (S_m^{exp}(t) - S_m^{mod}(t))^2 \, dt \right], \] \hspace{1cm} (7.7)

where \( N_e \) is the number of experiments, \( R_m \) the mean residual, \( t_{end}^{(m)} \) is the experimental duration, \( S_m^{exp} \) and \( S_m^{mod} \) are the experimental and calculated supersaturation values, respectively, all referred to the \( m \)-th experiment. Once the growth kinetics of a given system is determined, the induction time data can be combined with the growth kinetics to calculate the nucleation kinetics as described below.

Generally, the induction time may be defined as the period of time between the attainment of the nominal supersaturation and the detection of particles in the given system with a specific in-situ instrument. Since the amount of consumed supersaturation due to particle formation and growth mechanisms during the induction time is small, one can assume a constant supersaturation level during the induction time. For experiments at constant temperature, which is indeed the case in this work,
this results in constant rates of primary nucleation and growth during the induction time. In this case, we can integrate the following moment equations:

\[
\frac{dm_0}{dt} = J \tag{7.8}
\]

\[
\frac{dm_k}{dt} = kGm_{k-1}, (k = 1, 2, \ldots) \tag{7.9}
\]

between time zero and the induction time \( t_i \); \( m_0 \) and \( m_k \) denote the zeroth and k-th moment of the PSD, respectively, which are defined as:

\[
m_k = \int_0^\infty nL^kdL. \tag{7.10}
\]

Since the nucleation rate \( J \) and the growth rate \( G \) are constant during the induction time the integration of Eqs. 7.8 and 7.9 between 0 and \( t_i \) yields:

\[
m_0 = Jt_i \tag{7.11}
\]

\[
m_k = \frac{JG^k}{k+1} t_i^{k+1}, (k = 1, 2, \ldots). \tag{7.12}
\]

The induction time \( t_i \) can be viewed as the time when the volume fraction of the new solid phase in the crystallizer, i.e. \( k_v m_3 \), reaches the value of the minimum detectable volume fraction, \( \alpha_v \), which is specific for the detector and substances used in the experiment. Combining this definition with Eq. 7.12 in the case \( k = 3 \) yields the following functional relationship between the nucleation rate \( J \) and the induction time \( t_i \):

\[
J = \frac{4\alpha_v}{k_v G^3 t_i^4}. \tag{7.13}
\]

The calculated nucleation rates are linearly dependent on the factor \( \alpha_v \). Since this factor is assumed to be constant for a specific system, the comparison of the simulated and experimental PSD of the precipitate can be used to validate the determined nucleation and growth kinetics.
7.4 PDI 747 growth kinetics

The growth kinetics of PDI 747 was measured independently based on the procedure presented in section 7.2.4. First, the preparation and characterization of PDI 747 seed crystals is described in section 7.4.1. In the following, the experimental results and optimized kinetics parameters are presented in section 7.4.2.

7.4.1 Preparation and characterization of seed crystals

Two different seed fractions of PDI 747 crystals were produced by antisolvent precipitation at an initial supersaturation of $S_0 = 2.8$ and subsequent triple wet-sieving using three sieves with nominal mesh sizes of 64, 125, and $355 \mu m$ (Fritsch, Idar-Oberstein, Germany). The crystals of the sieve fraction larger than $355 \mu m$ mainly consisted of aggregates that could not be redispersed easily and were therefore discarded. The remaining seed crystal fractions in the size ranges of 64 to $125 \mu m$ and 125 to $355 \mu m$, were washed, filtered, and dried and are named F1 and F2 throughout this chapter. Fig. 7.9 shows the linear mass density PSDs of F1 and F2 which were characterized using the Coulter Multisizer 3 as described in section 7.2.3. While F1 features a narrow distribution with a mean size of about $50 \mu m$, fraction F2 has a slightly larger mean size of $70 \mu m$ and a broader distribution. The inset in Fig. 7.9 shows two SEM microphotographs of the two seed fractions; both images were taken at the same magnification. It can be observed, that F2 consists of slightly larger crystals compared to F1 and that both fractions feature mainly single crystals and small aggregates. The polymorphic form of the seed crystals was ascertained using X-ray powder diffraction as described in section 7.2.

7.4.2 Experimental results

Five seeded batch desupersaturation experiments were performed at three different initial supersaturation values to obtain an estimate of
Figure 7.9: Experimental PSDs of the two seed fractions F1 and F2. The inset displays the corresponding SEM photomicrographs. It can be readily observed that both fractions consist of needle-shaped crystals and small agglomerates.

PDI 747 growth kinetics. The experimental runs were labeled 1 to 5 and the corresponding experimental conditions are listed in Table 7.1. Based on this set of experiments the growth kinetics were estimated. Crystal growth is generally described as a combination of diffusion and a reaction step, which consists of the integration of the molecule in the crystal lattice. Therefore, crystal growth can be either diffusion limited, or integration controlled, or both. To check whether the growth kinetics was controlled by diffusional limitations, the mass transfer coefficient $k_d$ was estimated using the Sherwood correlation [6]:

$$k_d = \frac{D}{L} \left( 2 + 0.8 \left( \frac{\bar{c} L^4}{\nu^3} \right)^{1/5} S_{C^{1/3}} \right),$$

(7.14)
where $D$ is the diffusivity, $L$ the crystal size, $\bar{\epsilon}$ the average power input, $\nu$ the kinematic viscosity and $Sc$ the Schmidt number, i.e. $Sc = \nu / D$. With estimated values of $D = 2 \times 10^{-9} \text{ m}^2/\text{s}$, $L = 1 \times 10^{-4} \text{ m}$, $\bar{\epsilon} = 5.9 \times 10^{-2} \text{ W/kg}$, and $\nu = 1 \times 10^{-6} \text{ m}^2/\text{s}$ the mass transfer coefficient $k_d$ was calculated to be $2.2 \times 10^{-4} \text{ m}^2/\text{s}$. This mass transfer coefficient was used with the following equation to calculate diffusion controlled crystal growth rates [6]:

$$G_{\text{diff}} = k_d \frac{k_a}{3k_v \rho_c} \Delta c,$$

(7.15)

where $\rho_c$ is the crystal density, $\Delta c = c - c^*$ is the concentration based supersaturation, and $k_a$ and $k_v$ denote the surface and volume shape factors, respectively (for an aspect ratio of 10 $k_a = 0.42$ and $k_v = 0.01$). As a result, the diffusion limited growth rates were calculated as a function of supersaturation, yielding values of $G_{\text{diff}} = 2 \times 10^{-6}$ to $2 \times 10^{-4} \text{ m/s}$ for supersaturation ratios of $S = 1.1$ to $S = 11$.

To estimate the growth kinetics in the case of integration controlled growth, two different growth mechanisms were compared, i.e., the surface nucleation based birth and spread (B+S) mechanism [193] and the screw dislocation mechanism described by Burton, Cabrera and Frank (BCF) [7]. The following equations are used to describe the B+S and BCF mechanisms [164]:

$$G_{\text{B+S}} = A_{\text{B+S}} (S - 1)^{5/6} \exp(-B_{\text{B+S}}/(S - 1))$$

(7.16)

$$G_{\text{BCF}} = C_{\text{BCF}} \left((S - 1)^2 / D_{\text{BCF}}\right) \tanh(D_{\text{BCF}}/(S - 1)) .$$

(7.17)

To determine which of the two growth mechanisms describes the growth of PDI 747 more accurately, the experimental desupersaturation data were used together with the PBE model and the optimization algorithm described in section 7.3 to estimate the optimal parameter sets for both integration controlled growth correlations.

The following values were obtained for the B+S mechanism of Eq. 7.16:

$$A_{\text{B+S}} = 1.51 \times 10^{-7} \text{ m/s}$$

(7.18)

$$B_{\text{B+S}} = 1.01$$

(7.19)
Table 7.1: Experimental conditions of the desupersaturation experiments and corresponding mean residual values of the two growth correlations as described in section 7.4.2.

<table>
<thead>
<tr>
<th>Run</th>
<th>$S_0$</th>
<th>Seed</th>
<th>Seed mass</th>
<th>Mean residual (BCF)</th>
<th>Mean residual (B+S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1</td>
<td>F2</td>
<td>1.2</td>
<td>$4.3 \times 10^{-3}$</td>
<td>$1.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>F2</td>
<td>1.2</td>
<td>$3.8 \times 10^{-3}$</td>
<td>$0.8 \times 10^{-3}$</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>F2</td>
<td>1.2</td>
<td>$4.1 \times 10^{-3}$</td>
<td>$1.8 \times 10^{-3}$</td>
</tr>
<tr>
<td>4</td>
<td>3.2</td>
<td>F2</td>
<td>1.2</td>
<td>$4.4 \times 10^{-3}$</td>
<td>$1.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>5</td>
<td>2.6</td>
<td>F1</td>
<td>0.2</td>
<td>$7.3 \times 10^{-3}$</td>
<td>$1.2 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Changing the initial values of the estimated parameters over several orders of magnitude in the optimization procedure always produced the same results, hence indicating a global optimum. To evaluate the quality of the optimization results, the mean residuals were calculated for both growth mechanisms and each experiment and are shown in Table 4.1. It is worth noting, that the mean residuals calculated for the B+S mechanism varied between $0.8 \times 10^{-3}$ and $1.8 \times 10^{-3}$, while with the BCF mechanism the residuals were between twice and five times larger. Although the B+S correlation provides a better description of the experimental data than the BCF correlation in this supersaturation range more detailed experimental evidence such as AFM microphotographs would be needed to identify with certainty the growth mechanism of PDI 747 crystals.

However, the comparison of the experimental results with the estimated diffusion limited growth rates given above allows reaching the conclusion that the crystal growth of PDI 747 is integration controlled under the conditions studied in this work. Fig. 7.10 illustrates the good agreement between experimental and optimized simulated desupersaturation
curves according to B+S correlation for Runs 2, 4, and 5. Additionally, the final PSD of every growth experiment was measured and compared to the simulated PSD of the optimized growth kinetics. Figs. 7.11 and 7.12 show the seed PSD together with the final simulated and experimental PSD of Runs 2 and 5, respectively. It can be readily observed that in both experiments, the experimental PSD is significantly broader and shifted to larger particle size values compared to the simulated PSDs. Such results indicate particle agglomeration which was verified by SEM microphotographs, shown as insets in Figs 7.11 and 7.12.

Additionally, the final PSD of every growth experiment was measured and compared to the simulated PSD of the optimized growth kinetics. Figs. 7.11 and 7.12 show the seed PSD together with the final simulated and experimental PSD of Runs 2 and 5, respectively. It can be
Figure 7.11: PSD of the seed population F2 together with the experimental and simulated final PSD of Run 2. The inset displays a SEM photomicrograph of a single agglomerate at the end of the growth experiment.

readily observed that in both experiments, the experimental PSD is significantly broader and shifted to larger particle size values compared to the simulated PSDs. Such results indicate particle agglomeration which was verified by SEM microphotographs, shown as insets in Figs 7.11 and 7.12.

7.5 PDI 747 nucleation kinetics

The results of induction time measurements of PDI 747 as described in section 7.2.5 are combined with the growth kinetics determined in section 7.4 to yield nucleation kinetics. The section is organized as follows. First, the experimental results are presented in section 7.5.1. Then, the esti-
Figure 7.12: PSD of the seed population F1 together with the experimental and simulated final PSD of Run 5. The inset displays a SEM photomicrograph of a single agglomerate similar at the end of the growth experiment.

Information of the nucleation kinetics of PDI 747 is presented and discussed in section 7.5.2.

7.5.1 Induction time measurements

A series of induction time experiments was performed at various values of the supersaturation $S$ as described in section 7.2.5. The measured induction times are shown as a function of initial supersaturation in Fig. 7.13. The measured values exhibit some scattering for low supersaturation values. However, the expected decrease of induction time with increasing supersaturation can be observed. It is worth noting, that the experimental range for the initial supersaturation had to be limited to a
maximum of $S = 7.4$. For larger supersaturation values the experimental uncertainty increased significantly since the induction time is in the same order of magnitude as the mixing time. The set of averaged induction times will be used in the following section to estimate the nucleation rate.

### 7.5.2 Estimation of nucleation kinetics

The nucleation rate $J$ can be related to the independently measured induction times and growth kinetics $G$ as described by Eq. 7.13. The nucleation rate is linearly depending on two factors, namely the minimum detectable volume fraction $\alpha_v$ and the reciprocal of the volume shape factor $k_v$, respectively. Assuming that $\alpha_v$ and $k_v$ are constant for certain experimental conditions, $\alpha_v$ can be determined by comparison of the simulated and experimental PSDs of different precipitation experiments, and $k_v$ can be estimated via SEM microphotographs of the final
Figure 7.14: Scanning electron microphotograph of the final PDI747 particles precipitated at two different initial supersaturation values of $S = 5.5$ and $S = 6.3$.

Particles of PDI747. To determine these two parameters for PDI747, precipitation experiments were conducted at different initial supersaturation levels. Fig. 7.14 shows two SEM microphotographs of the final precipitate obtained by antisolvent precipitation at initial supersaturation values of $S = 5.5$ and $S = 6.3$, respectively. In both cases the precipitate contains agglomerates as well as broken crystal fragments. This indicates that besides nucleation and growth also other phenomena, such as agglomeration and particle breakage, have taken place during the experiment at these operating conditions. Consequently, the value of $k_v = 0.01$ that we have chosen is only representative of a rather broad range of values that occur in reality.

Then, also the value of $\alpha_v$ could be determined by comparing the simulated and experimental PSDs of the precipitation experiments. Fig. 7.15 shows the experimental PSD of a precipitation experiment with an initial
supersaturation of $S = 6.3$ and the corresponding simulated PSDs for three different $\alpha_v$ values, namely $1 \times 10^{-2}$, $5 \times 10^{-3}$, and $1 \times 10^{-2}$. Higher values than $\alpha_v = 1 \times 10^{-2}$ were not considered to be reasonable, since the maximum solid fraction at the end of the experiments is about $4 \times 10^{-2}$, as calculated through material balances, and the induction times were detected at the beginning of particle formation, thus at much lower values of the solid fraction than $4 \times 10^{-2}$. A value of $\alpha_v = 1 \times 10^{-3}$ was assumed to be the lower limit due to a comparison to the $\alpha_v$ estimated in chapter 5 in the case of the nucleation of L-glutamic acid, where $\alpha_v$ was estimated to be $4 \times 10^{-3}$. In Fig. 7.15 it can be observed that all simulated PSDs are significantly narrower than the experimental PSD. This indicates additional mechanisms besides nucleation and crystal growth,
Figure 7.16: Nucleation rate of PDI747 as a function of supersaturation plotted as (ln(J) vs ln^{-2}(S)). The different nucleation regimes are highlighted and the corresponding kinetic parameters are indicated.

such as agglomeration and breakage, as already indicated by the SEM microphotographs of Fig. 7.14. On the basis of these experiments one cannot characterize precisely α_v and hence the nucleation kinetics of PDI747, due to the fact that agglomeration and breakage alter the final PSD. These effects have not been characterized and are not considered in the precipitation model yet. Therefore, the nucleation rates can only be given here for an approximated value of α_v. The nucleation kinetics of PDI747, calculated using Eq. 7.13 and assuming α_v = 5 \times 10^{-3}, can be determined by interpolation of the experimental data with a straight line when plotting ln(J) over ln^{-2}(S), as expected from classical nucleation theory. As a matter of fact two regimes are observed, as shown in Fig. 7.16, which correspond to homogeneous and heterogeneous nucleation. The following kinetic equations for homogeneous and heterogeneous nu-
cleation were obtained:

\[ J_{Hom}(S) = 6.8 \times 10^{18} \exp \left( \frac{-71}{\ln^2 S} \right) \]  
\[ J_{Het}(S) = 1.7 \times 10^{11} \exp \left( \frac{-13}{\ln^2 S} \right). \]

The overall nucleation rate is at any supersaturation level the sum of these two contributions, i.e.

\[ J(S) = J_{Hom}(S) + J_{Het}(S). \]

### 7.6 Conclusions

In this work two characterization methods for nucleation and crystal growth have been applied to the antisolvent precipitation of PDI747. The growth kinetics was determined based on seeded batch desupersaturation experiments by combination of a population balance model and a parameter optimization routine. The solute concentration was measured using ATR-FTIR spectroscopy. During these experiments conditions have been chosen so as to keep at a minimum mechanisms such as nucleation, agglomeration and breakage. FBRM, Coulter multisizer and scanning electron microscopy have been used to check this.

Then, in a series of precipitation experiments, i.e. including both nucleation and growth, the induction time was measured as a function of supersaturation using ATR-FTIR and FBRM. Plotting the calculated nucleation rates according to the functional form of the classical nucleation theory allows for the estimation of the nucleation rate parameters, as shown already in the case of L-glutamic acid in chapter 5. This approach has been applied also in this case, where however the accuracy of the nucleation rate estimate is limited by the difficulty of assigning a value to the threshold of the solid phase volume fraction beyond which particles are detected. Moreover, contrary to the case of growth experiments, agglomeration and breakage play a bigger role in the precipitation experiments, thus reducing the accuracy of the model used here.
the limits of the assumptions made in this study, we have been able to estimate nucleation rates in a broad supersaturation range, wherein two nucleation regimes, i.e., homogeneous and heterogeneous nucleation, have been identified.
Chapter 8

Concluding remarks

In the pharmaceutical and fine chemical industries precipitation processes are mainly employed to meet the increasing demand for micron and sub-micron sized organic solid substances with defined particle size distribution, controlled polymorphic form, and high levels of purity and yield. Yet, rigorous process design and scale-up from laboratory to production scale require the sound knowledge of the underlying particle formation mechanisms and of the concurrent mixing processes in the reactor. Conceptually, this thesis aims at two things: firstly, the use of current in situ process analytical technologies (PAT) for the development of robust and fast characterization methods to determine nucleation and growth kinetics; secondly, to combine the experimentally determined particle formation and growth kinetics with population balance modeling to yield a simulation tool of the precipitation process. Finally, the accuracy of the determined kinetics correlations can be estimated by comparing the experimental and simulated final PSDs for different initial conditions. Two model systems, namely L-glutamic acid and PDI 747, were studied and the particle formation and growth kinetics during pH-shift and antisolvent precipitation were characterized.

A reliable and robust characterization method for nucleation kinetics and its application to the pH-shift precipitation of L-glutamic acid was presented in Chapter 3. The method allowed for the fast estimation of
nucleation rates based on the combination of two in situ analytical technologies, namely ATR-FTIR and FBRM to determine induction time as a function of supersaturation. On the one hand, the application of ATR-FTIR was used to obtain precise liquid phase information, i.e., about the concentrations of the different L-glutamic acid species in solution as a function of pH. Thus, ATR-FTIR could be used to monitor the mixing behavior of the liquid phase and to determine accurately the time interval needed until supersaturation was homogeneously established throughout the reactor. Therefore, ATR-FTIR not only identified precisely the starting point of the induction time but allowed also for the determination of the mixing time in the reactor. This is of particular importance in order to judge when the measurement technique is applicable, which is the case when the mixing time is short compared to the determined induction time. On the other hand, FBRM was used to obtain in situ solid phase information, i.e., to detect the onset of particle formation in a reproducible manner. Finally, the experimental information of ATR-FTIR and FBRM can be combined to determine the induction time as a function of supersaturation. It can be assumed that the rates of particle formation and growth are constant during the induction time since the level of supersaturation is constant, which was proved using the ATR-FTIR. Thus, the nucleation rate can be directly calculated from induction time data once the corresponding growth kinetics are known. One uncertainty in the nucleation rate calculations is introduced by the factor of the detectable solid fraction $\alpha_v$, which was assumed to be device and system dependent. With estimations for the growth kinetics and $\alpha_v$ the nucleation kinetics of L-glutamic acid were calculated. Therefore, induction time experiments can be used to determine nucleation rates in stirred batch reactors combining ATR-FTIR and FBRM up to supersaturation levels where the precipitation process is not influenced by mixing effects.

Chapter 4 is devoted to the development of a characterization method for the independent measurement of L-glutamic acid growth kinetics. Again, the method is based on the same in situ process analytical technologies that were used in Chapter 3 for the measurement of induction times, namely ATR-FTIR spectroscopy and FBRM. ATR-FTIR was used to monitor the solute concentration whereas the FBRM data allowed to
check whether the required absence of other phenomena like nucleation, agglomeration or breakage was fulfilled. A Coulter particle analyzer employing the electric zone sensing method was used to characterize accurately the initial PSD of the different seed fractions. The growth kinetics can be determined independently from other mechanisms by measuring the solute concentration as a function of time in seeded batch experiment and combining the experimental data with the particle size distribution of the seed crystals and population balance modeling. Three different experimental parameters, i.e., initial supersaturation, seed size, and seed mass, were varied to check for the robustness of the determined growth kinetics and the absence of size dependent growth. It could be shown, that this characterization method is robust also in case of occurring agglomeration. The growth mechanism was identified as birth and spread in agreement with literature and the comparison of the determined growth kinetics to correlations at different temperatures proposed by other authors has shown reasonable agreement.

In Chapter 5, the L-glutamic acid growth kinetics determined in Chapter 4 were combined with the induction time measurements presented in Chapter 3 to calculate more accurate nucleation kinetics. Then, a population balance based process model was introduced, which combined the newly determined nucleation and growth kinetics to simulate the precipitation of L-glutamic acid via pH-shift. It could be shown, that the comparison of simulated and final PSDs served for two different purposes. Firstly, it allowed for the determination of the value for \( \alpha_v = 3 \times 10^{-2} \), i.e., the detectable volume fraction used in the previous chapter 3 to calculate nucleation rates from induction time data. This was a valuable information since the order of magnitude of \( \alpha_v \) was based until then on estimates obtained from other model systems, e.g., glass and ceramic spheres. Secondly, the comparison of experimental and simulated PSDs indicated that secondary mechanisms, in particular agglomeration, had to be taken into account since the experimentally obtained L-glutamic acid PSD featured significantly larger particles than the simulation results. This assumption could be validated using SEM photomicrographs. Consequently, further experimental work is necessary to describe agglomeration kinetics in order to obtain a complete process model of L-glutamic acid precipitation. Yet, the process model was used
together with homogeneous and heterogeneous nucleation rates and the measured growth kinetics to simulate the dependence of the final PSD on the initial supersaturation during L-glutamic acid precipitation.

In the chapters 3 and 4, nucleation and growth kinetics of the metastable α polymorph of L-glutamic acid were determined using specific characterization methods based on ATR-FTIR and FBRM. Chapter 6 is devoted to the determination of nucleation and growth kinetics of both polymorphs of L-glutamic acid, i.e., the metastable α form and the stable β form. Four different in situ analytical instruments, namely ATR-FTIR spectroscopy, Raman spectroscopy, FBRM, and PVM were combined to monitor the solvent-mediated polymorphic transformation of L-glutamic acid during unseeded and seeded processes at 45°C. The higher temperature than in the previous experiments described in Chapters 2 and 3 was chosen to accelerate the polymorphic transformation velocity. The combination of in situ measurement data provided by the different tools allowed for the identification of the fundamental phenomena that govern the transformation process. Moreover, the characteristic process information from the analytical data was used to estimate the nucleation and growth kinetics of both polymorphs in the population balance model. Experiments seeded with the metastable polymorph were found particularly useful to decouple the kinetics of the two polymorphs and determine the kinetics parameters of the stable β form. Finally, a simulation study allowed for the conclusion that the surface nucleation of the stable β form on the surface of the metastable α crystals is the rate controlling step of the transformation process.

Chapter 7 describes the application of the nucleation and growth kinetics characterization methodologies developed in Chapters 3 and 4 to the antisolvent precipitation of PDI747, an organic compound with the molecular weight of a typical active pharmaceutical ingredient. Firstly, a small set of seeded batch desupersaturation experiments was monitored using ATR-FTIR and the experimental data was combined with PBE modeling to yield the growth kinetics. The absence of other mechanisms than growth was checked using in situ FBRM, an off line Coulter particle analyzer and scanning electron microscopy. Despite the high aspect ratio of PDI747 crystals of about 10 the breakage of particles was not found to be significant during the growth experiments. The induc-
tion time was measured as a function of supersaturation as described in Chapter 3 by combining ATR-FTIR and FBRM. Finally, the nucleation rates of PDI747 were calculated by combining growth kinetics and induction time data. Plotting the calculated nucleation rates according to the functional form of the classical nucleation theory allowed for the identification of two nucleation regimes, i.e., homogeneous and heterogeneous nucleation together with the corresponding parameters in the expression for the nucleation rate. However, the accuracy of the nucleation rate estimate was limited by the difficulty of assigning a value to the threshold of the solid phase volume fraction beyond which particles are detected. This was mainly due to secondary phenomena such as agglomeration and breakage, which played a bigger role in the precipitation of the needle-like PDI747 as compared to the PDI747 growth experiments or the precipitation of the rhombic α L-glutamic acid crystals. Consequently, this resulted in reducing the accuracy of the model used here. Thus, it was concluded that a complete process model of the PDI747 precipitation requires further work characterizing the kinetics of the underlying mechanisms of agglomeration and particle breakage.

Thus summarizing, the combination of in situ analytical tools and mathematical modeling allowed for faster and more robust nucleation and growth kinetics characterization during precipitation processes. In particular Chapter 7 has shown, that already a small number of growth characterization experiments can yield accurate growth kinetics. Consequently, such an approach seems suitable to significantly improve the development of precipitation processes of polymorphic compounds in the future, particularly in the pharmaceutical industry.

Despite these promising results regarding nucleation and growth, the present work indicates the need for future research, in particular in the field of agglomeration and breakage. Both model compounds showed agglomeration effects, and a suitable characterization method for agglomeration kinetics should be sought to improve the quality of simulation results. Moreover, other secondary effects such as secondary nucleation and particle breakage might become important for other systems, thus inducing the need to characterize the corresponding kinetics. However, as more and more kinetics of different fundamental mechanisms are characterized, the easier it will be to estimate the kinetics of remaining sec-
Another critical issue is the characterization of precipitation processes at such high levels of supersaturation that they become influenced by mixing effects. The particle formation mechanisms of these processes cannot be studied in conventional stirred batch reactors since liquid phase mixing and particle formation occur concurrently. Although such mixing controlled precipitations were not studied in this thesis, experimental facilities can be devised as described in Chapter 2.1.2 that are able to achieve smaller characteristic mixing times than the corresponding particle formation mechanisms. Consequently, such an experimental set-up will allow for the separation of mixing and particle formation steps thus allowing for the characterization of the primary homogeneous nucleation rate at very high supersaturation levels. Ultimately, this will allow for the comparison of nucleation kinetics determined in at higher and lower supersaturation levels in different experimental facilities.

This work has resulted in laying the groundwork for the development of characterization methods of nucleation and growth during precipitation processes based on in situ measurement tools. Besides the research needed to characterize agglomeration effects described above, the application of the presented methods on a broader scale for the characterization of nucleation and growth kinetics for different organic compound classes will help to evaluate the applicability of the presented methods in the pharmaceutical and fine chemical industries.
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