Doctoral Thesis

In situ monitoring and modeling of crystallization processes

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In situ monitoring and modeling of crystallization processes

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presented by

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Abstract

Crystallization from solution is among the most frequently employed separation processes to obtain solid material with a high purity and is widely applied in the production of fine chemicals and pharmaceuticals. Identification and characterization of the different kinetic phenomena in crystallization processes, i.e., nucleation, growth and dissolution, are essential for process understanding, allow process modeling and enable process design, optimization and control. Apart from particle size characterization techniques, such as in situ microscopy and laser (back) scattering techniques, spectroscopic tools provide detailed time-resolved information about a given crystallization process. Among others, in situ infrared and Raman spectroscopy are both recognized as essential techniques enabling the estimation of solid as well as of liquid phase compositions.

The quantitative application of both spectroscopic techniques usually involves multivariate calibration approaches such as principal component regression and partial least squares regression to overcome numerical problems due to collinearity. One of the objectives of this thesis is to demonstrate the quantitative application of in situ infrared and Raman spectroscopy to crystallization processes through these multivariate approaches. Protocols to obtain accurate and robust calibration models for both spectroscopic techniques are presented and discussed.

However, it is evident that calibration is time-consuming and more sophisticated calibration-free quantitative approaches to apply in situ infrared and in situ Raman spectroscopy are desired. This thesis demonstrates that both spectroscopic techniques can be applied quantitatively without extensive calibration efforts. This novel calibration-free approach is discussed and tested thoroughly through its application to several crystallization processes. First, relevant kinetics of L-glutamic acid’s solvent-mediated polymorph transformation in water are elucidated using in situ Raman spectroscopy combined with a calibration and a process model. Then, it is demonstrated that the secondary
nucleation rate parameters can also be obtained by fitting the measured time-resolved Raman spectra directly, thereby avoiding calibration efforts. Second, the crystal growth rate of paracetamol in water is studied using seeded batch desupersaturation experiments monitored using in situ infrared and in situ Raman spectroscopy. It is demonstrated that by fitting the measured time-resolved infrared spectra directly and by fitting the desupersaturation profiles obtained from these infrared spectra combined with a calibration model, essentially the same crystal growth rate parameters can be estimated. Very similar growth rate parameters can be obtained by fitting the measured time-resolved Raman spectra. Therefore, this thesis shows that in the case a descriptive process model is available, both in situ spectroscopic techniques can be applied in a calibration-free manner, thereby avoiding time-consuming and costly calibrations.
Zusammenfassung


Table of Contents

1 Introduction
   1.1 Process Analytical Technologies .......................... 2
   1.2 Objectives and structure of this thesis .................. 4

2 Experimental
   2.1 Experimental setup ........................................ 6
   2.2 In situ techniques ......................................... 7
      2.2.1 ATR-FTIR spectroscopy ................................. 7
      2.2.2 Raman spectroscopy .................................... 9
      2.2.3 FBRM .................................................. 12
      2.2.4 In situ imaging ........................................ 13
   2.3 Ex situ techniques .......................................... 14
      2.3.1 Powder X-ray diffraction .............................. 14
      2.3.2 Multisizer ............................................. 14
      2.3.3 Microscopy ............................................. 15
3 Modeling

3.1 Modeling spectroscopic data ..................................... 16
   3.1.1 Calibration-based approaches .............................. 18
   3.1.2 Calibration-free approaches ............................... 26

3.2 Process modeling .................................................. 28
   3.2.1 Population balance equation .............................. 28
   3.2.2 Solution of the population balance equation .......... 29
   3.2.3 Constitutive equations ...................................... 34
   3.2.4 Illustrative example ....................................... 36

3.3 Parameter estimation .............................................. 38

4 Quantitative application of in situ ATR-FTIR and Raman spectroscopy

4.1 Introduction ...................................................... 40

4.2 Experimental ...................................................... 42
   4.2.1 Materials and methods .................................... 42
   4.2.2 Experimental design ...................................... 43
   4.2.3 Multivariate data analysis ............................... 50

4.3 Results and discussion ........................................... 50
   4.3.1 ATR-FTIR spectroscopy .................................. 50
   4.3.2 Raman spectroscopy ...................................... 57

4.4 Conclusions ....................................................... 65
## Monitoring and modeling the polymorph transformation of L-glutamic acid

5.1 Calibration-based approach ................................. 70
   5.1.1 Introduction ......................................... 70
   5.1.2 Materials and methods ................................. 72
   5.1.3 Mathematical description of the polymorph transformation ......................................... 77
   5.1.4 Results and discussion ................................. 82
   5.1.5 Conclusions ........................................... 96

5.2 Calibration-free approach ................................. 97
   5.2.1 Introduction ......................................... 97
   5.2.2 Modeling the Raman data ............................... 100
   5.2.3 Modeling the polymorph transformation .......... 103
   5.2.4 Results and discussion ............................... 105
   5.2.5 Conclusions ........................................... 114

## Estimating growth rates calibration-free using in situ spectroscopy

6.1 Introduction ........................................... 117

6.2 Materials and methods .................................. 119
   6.2.1 Materials ........................................... 119
   6.2.2 Experimental setup .................................. 120
   6.2.3 Characterization techniques ......................... 121
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.4</td>
<td>Experimental procedure</td>
<td>121</td>
</tr>
<tr>
<td>6.3</td>
<td>Modeling the spectroscopic data</td>
<td>122</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Estimating the model parameters</td>
<td>122</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Parameter estimation techniques</td>
<td>125</td>
</tr>
<tr>
<td>6.4</td>
<td>Modeling the crystallization process</td>
<td>126</td>
</tr>
<tr>
<td>6.5</td>
<td>Results and discussion</td>
<td>129</td>
</tr>
<tr>
<td>6.5.1</td>
<td>Fitting measured desupersaturation profiles</td>
<td>130</td>
</tr>
<tr>
<td>6.5.2</td>
<td>Fitting the measured time-resolved spectra</td>
<td>132</td>
</tr>
<tr>
<td>6.5.3</td>
<td>Comparison of different modeling approaches</td>
<td>135</td>
</tr>
<tr>
<td>6.5.4</td>
<td>Comparison with literature data</td>
<td>137</td>
</tr>
<tr>
<td>6.6</td>
<td>Conclusions</td>
<td>139</td>
</tr>
<tr>
<td>7</td>
<td>The direct inverse method: a new method to</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>estimate isotherm parameters</td>
<td></td>
</tr>
<tr>
<td>7.1</td>
<td>Introduction</td>
<td>141</td>
</tr>
<tr>
<td>7.2</td>
<td>Experimental</td>
<td>144</td>
</tr>
<tr>
<td>7.2.1</td>
<td>System</td>
<td>144</td>
</tr>
<tr>
<td>7.2.2</td>
<td>Chemicals</td>
<td>144</td>
</tr>
<tr>
<td>7.2.3</td>
<td>Column</td>
<td>145</td>
</tr>
<tr>
<td>7.3</td>
<td>Modeling the spectroscopic data</td>
<td>145</td>
</tr>
<tr>
<td>7.3.1</td>
<td>Notation</td>
<td>145</td>
</tr>
<tr>
<td>7.3.2</td>
<td>Classical inverse method</td>
<td>146</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>7.3.3</td>
<td>Direct inverse method</td>
<td>147</td>
</tr>
<tr>
<td>7.3.4</td>
<td>Parameter estimation techniques</td>
<td>148</td>
</tr>
<tr>
<td>7.4</td>
<td>Chromatographic model</td>
<td>149</td>
</tr>
<tr>
<td>7.5</td>
<td>Results and discussion</td>
<td>150</td>
</tr>
<tr>
<td>7.5.1</td>
<td>Simulation results</td>
<td>150</td>
</tr>
<tr>
<td>7.5.2</td>
<td>Experimental results</td>
<td>153</td>
</tr>
<tr>
<td>7.5.3</td>
<td>Discussion</td>
<td>161</td>
</tr>
</tbody>
</table>

8 Concluding remarks  163

References  169

A List of symbols  186

B List of abbreviations  188

C List of Figures  190

D List of Tables  201
Chapter 1

Introduction

Crystallization refers to the formation of a crystalline solid material from a solution, melt, amorphous solid or vapor. The formation of crystalline particles is mainly governed by two mechanisms, i.e. nucleation and crystal growth. During nucleation, the molecules self-organize into stable clusters on a nanometer scale. Crystal growth refers to the subsequent attachment of molecules to the crystal surfaces and the integration into the crystal\(^1\).

Crystallization from solution is one of the most important separation processes to obtain solid material with a high purity and is widely applied in the production of fine chemicals and pharmaceuticals where an estimated 90 % of all products is delivered in a crystalline form\(^2-^4\). The two main reasons for its wide application in these industries are a high purity and an improved chemical stability of the final crystalline product.

The driving force of all crystallization processes is the supersaturation, i.e. the difference between the actual and equilibrium solute concentra-
tion, and it can be achieved in various ways, e.g. by cooling, by addition of an antisolvent that significantly decreases the solubility or by chemical reaction as it is done in pH-shift crystallization.

To a certain extend the outcome of a crystallization process, e.g. the crystal size, the crystal shape, the crystallinity or the polymorphic form, can be manipulated by changing the process variables such as the cooling rate or the rate of antisolvent addition. The optimal set of operating conditions that results in the desired product properties can be found through a large number of experiments and applying a trial and error approach. A more intelligent and robust approach is to formulate a mathematical description of the process and to estimate the unknown model parameters through a smaller number of experiments. The established process model can then be applied to predict the outcome of a crystallization process and to obtain the desired product properties by adjusting the process variables. In this context, it is essential to elucidate the governing kinetic phenomena and to estimate the unknown parameters that appear in their expressions. Ultimately, accurate characterization of the different kinetic phenomena in crystallization, i.e. nucleation, growth and dissolution, allow process modeling and enable process design, optimization and control.

1.1 Process Analytical Technologies

One way to estimate the unknown model parameters is to compare the simulated or modeled time-evolution of the process variables to the true time-evolution of these process variables that is obtained through experiments. In the case of crystallization, these process variables include the particle size distribution, the solute concentration and the concentration
of crystals. By tuning the model parameters, the difference between the modeled and the true process variable, e.g. the time-evolution of the solute concentration, can be minimized and the best estimates for the model parameters can be obtained.

One method to obtain the true time-evolution of a certain process variable such as the solute concentration, is to draw samples from the reactor and to determine their concentration values using another characterization technique such as high-pressure liquid chromatography (HPLC). This classical approach has proven to be effective in many cases but requires a significant amount of experimental work. Furthermore, the sample composition might change during the time period from drawing the sample to analyzing it.

In situ measurement techniques or process analytical technologies (PATs), enable measuring the process variables of interest in the reactor itself and eliminate the necessity of sampling and related experimental efforts. In situ microscopy and laser (back) scattering techniques enable in situ measurement of the particle size distribution thereby provide detailed time-resolved information about a given crystallization process. Among others, in situ infrared and in situ Raman spectroscopy are both recognized as essential techniques enabling the estimation of solid as well as of liquid phase compositions thereby allowing process understanding and enabling process modeling.

In the recent years, in situ measurement techniques have been applied more and more frequently to crystallization processes, in academia as well as in industry\textsuperscript{4-7}. This work addresses the application of in situ infrared and in situ Raman spectroscopy to crystallization processes and how these characterization techniques can be applied efficiently to estimate unknown kinetic parameters.
1.2 Objectives and structure of this thesis

The objective of this thesis is to demonstrate the quantitative application of in situ infrared and Raman spectroscopy through multivariate approaches. On the one hand, there is the need for protocols to obtain accurate and robust calibration models for both spectroscopic techniques. On the other hand, calibration is time-consuming and more sophisticated calibration-free quantitative approaches to apply in situ infrared and in situ Raman spectroscopy are desired.

In Chapter 2, all experimental characterization techniques, in situ as well as ex situ techniques, are described in detail. Chapter 3 discusses the theoretical methods that will be used in the subsequent chapters, i.e. different approaches to translate the measured spectroscopic data into concentration values as well as different modeling approaches to describe crystallization processes. Chapter 4 describes the quantitative application of in situ infrared and in situ Raman spectroscopy to different crystallization processes through a calibration-based approach. In Chapter 5, L-glutamic acid’s polymorph transformation will be characterized through seeded and unseeded transformation experiments and using in situ Raman spectroscopy. Then it will be demonstrated that very similar kinetic parameters can be estimated using the same Raman data, but using a calibration-free approach that eliminates the time-consuming development of a calibration model for Raman spectroscopy. Chapter 6 addresses the estimation of crystal growth rates using in situ infrared and Raman spectroscopy in a calibration-free manner. The broad applicability of the calibration-free approach presented in the second part of Chapter 5 is demonstrated through its application to a chromatographic process in order to estimate adsorption isotherm parameters in Chapter 7. Finally, concluding remarks are given in Chapter 8.
A thorough understanding of a certain process can only be established through accurate observation of the process during a period of time. These observations are then used to describe a process in terms of a mathematical model that can be used to predict the time-evolution of the process under certain operating conditions. In this chapter, a description of the experimental setup and all characterization techniques that were used to obtain the results reported in this thesis will be given. First, the experimental setup in which all experiments have been performed will be described. Subsequently, different in situ monitoring techniques will be discussed followed by a brief description of more classical ex situ characterization techniques.
2. Experimental

2.1 Experimental setup

Experiments were performed in two jacketed reactors of 100 and 500 ml volume. Both reactors were temperature controlled using a Ministat 230-3 thermostat and a Pt 100 temperature sensor, and were equipped with a 4-blade glass impeller (LTS, Biel-Benken, Switzerland). Due to spatial restrictions, the 100 ml reactor allowed for the use of only one probe, i.e. the Raman immersion probe. In the 500 ml reactor, ATR-FTIR, Raman and FBRM measurements could be performed simultaneously; a schematic representation of the reactor is given in Figure 2.1. The small reactor was used to reduce the mass of seed crystals required for calibration measurements and for seeded crystallization experiments.

Figure 2.1: Experimental setup enabling simultaneous application of FBRM, Raman and ATR-FTIR spectroscopy.
2.2 In situ techniques

In situ monitoring techniques or process analytical technologies (PATs) enable real time measurement of process variables, e.g. the liquid composition, and thereby enable improved process understanding and process design\textsuperscript{5,8–10}. Vibrational spectroscopy techniques are among the most frequently used in situ monitoring techniques and in this work in situ infrared and in situ Raman spectroscopy were used extensively to characterize the evolution of liquid and solid phase compositions over time. Besides, laser (back) scattering and imaging techniques enable characterization of particle size and shape, a property that is crucial for downstream processing and affects also physical properties such as bioavailability.

2.2.1 ATR-FTIR spectroscopy

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy enables measurement of liquid phase compositions and has been applied widely in crystallization and precipitation studies\textsuperscript{11–18}. An infrared spectrometer employs light with wavelengths that range from 2.5 to 15 µm, i.e. polychromatic light, of which a portion is absorbed by the rotations and vibrations of the molecules in the substrate. Due to the low penetration depth, the ATR probe allows for exclusively liquid phase monitoring also in the presence of solid material. From the absorptions at characteristic wavenumbers, the molecular structure can in principle be elucidated, however that was not the application of ATR-FTIR spectroscopy in this work. In ATR spectroscopy, the light is led through an ATR-probe; depicted in Figure 2.2. At the end of this probe an optically dense crystal, e.g. a diamond, causes the beam to undergo total internal
reflection, thus creating an evanescent wave that propagates a very short distance (typically less then 5 µm) into the solution or the suspension in which the probe is immersed. As this evanescent wave travels through the solution, the molecules in the solution will absorb (attenuate) very distinct parts of the wide spectrum of the infrared light due to intra- and intermolecular vibration and rotations. The exact frequency of the light that is absorbed, and the extent to which it is absorbed, depends on the molecular structure of the compounds in the solution and their corresponding concentrations. As a result of this compound-specific attenuation, certain frequencies of the evanescent wave will have lost part of their intensity by the time they reach the end of the probe window and are led to the detector, where this decrease in intensity is recorded. In an FTIR spectrometer, this detector is an interferometer that, after performing a Fourier transformation, provides the infrared spectrum of the solution.

![Diagram](image)

**Figure 2.2:** The principle of ATR spectroscopy. A ray of light is internally totally reflected off the surface of the probe window. The evanescent wave created in this way travels a short distance away from the window where molecules in solution absorb part of the light.

The values of the absorptions at different wavenumbers can be related to the concentration of the different compounds in solution through the Beer-Lambert law that describes the relationship between the absorption of light and the concentration of compounds in the solution through
2.2 In situ techniques

which the light traveled:

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

(2.1)

where \( A \) is the absorbance, \( a \) is the molar 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.

In most cases, univariate calibrations based on eq. (2.1) suffice to relate the absorbance to a concentration of the compounds of interest. However, in case of time-dependent process variables such as temperature or solvent composition or in the case of overlapping signals due to multiple components, multivariate calibration approaches are required in order to apply ATR-FTIR spectroscopy in a quantitative manner.

Spectra were collected using a ReactIR 4000 system from Mettler-Toledo (Greifensee, Switzerland), equipped with a 11.75” DiComp immersion probe and a diamond ATR crystal. Spectra were collected over a 1 or 2 minute interval in the 650 – 4000 cm\(^{-1}\) region with a resolution of 2 cm\(^{-1}\) and were averaged over 128 scans.

2.2.2 Raman spectroscopy

Contrary to infrared spectroscopy, monochromatic light is used to irradiate a sample in Raman spectroscopy\(^{19}\). The Raman effect is due to the inelastic scattering of incident light. If a light quantum or a photon with energy \( h\nu_0 \) hits a molecule, light can be scattered elastically, i.e. the scattered photon has the same energy, or inelastically, i.e. the energy carried by the scattered photon has changed with respect to the incoming photon. The elastic scattering process has the highest probability
and is known as Raleigh scattering. However, at a lower probability also
the inelastic, so-called Raman scattering process, occurs and the result-
ing scattered energy quantum has an energy of $h\nu_0 \pm h\nu_s$, where $h\nu_s$ is
related to the molecular structure of the compound. The Raman scat-
tered light is frequency-shifted with respect to the excitation frequency
to lower or to higher frequencies resulting in Stokes or Anti-Stokes Ra-
man scattering, respectively.

The principle of Raman scattering is illustrated in Figure 2.3. At am-
ambient temperature most molecules are in their vibrational ground state.
According to Boltzmann’s law, a much smaller number of molecules is
in the vibrational excited state. Therefore, Raman scattering resulting
in a quantum with lower energy $h\nu_0 - h\nu_s$ has a higher probability than
the reverse process, i.e. emission of a quantum with higher energy corre-
ponding to $h\nu_0 + h\nu_s$. Therefore, the Stokes signal has a higher intensity
than the anti-Stokes signal as illustrated in Figure 2.3$^{19}$.

The Raman scattering effect is so feeble that only about one photon in
every $10^{12}$ incident photons is scattered inelastically. However, the use of
intense laser radiation and efficient photomultiplier detectors make this
technique viable. Typically, the lasers used in Raman spectrometers
emit light in the near-infrared range. Mostly, Raman spectrometers are
equipped with conventional CCD detectors or employ Fourier transform
to record the data. As in the case of infrared spectroscopy, Raman spec-
troscopy can be applied in situ by sending the light through a immersion
probe where the light interacts with a medium at the probe tip.

In this work, in situ Raman spectroscopy has been applied mainly
to monitor the solid phase composition in the case of crystal suspensions$^{20-25}$. However, since the Raman scattering effect results from both
the solid and the liquid phase, Raman spectroscopy can also be applied
for solute concentration monitoring.
2.2 In situ techniques

\[ \text{Figure 2.3: Principle of Raman scattering. (a) Quanta of energy } h\nu_0 \text{ interacts with the molecule (L-glutamic acid) resulting in inelastic scattering; (b) energy level diagram: irradiation with light quanta } h\nu_0 \text{ may result in scattering of quanta with energy } h\nu_R^+ = h\nu_0 + h\nu_s \text{ or } h\nu_R^- = h\nu_0 - h\nu_s, \text{ anti-Stokes and Stokes scattering, respectively; (c) simplified Raman spectrum, signal at } \nu_0 \text{ is due to Rayleigh scattering, signal at lower frequency (Stokes signal) has a higher intensity than the signal at higher frequency (Anti-Stokes signal).} \]
In this work, a RA 400 Raman spectrometer from Mettler-Toledo (Greifensee, Switzerland) equipped with a 250 mW frequency stabilized laser diode at 785 nm and a thermoelectrically cooled CCD detector was used. In situ measurements were recorded using a 5/8” ball type immersion probe from Inphotonics (Norwood, MA, USA) connected via a fiber optic (thickness of collection and excitation fibers were 100 \( \mu \text{m} \) and 200 \( \mu \text{m} \), respectively). Solid powder mixtures were analyzed using a flat immersion probe with identical characteristics as the probe used for suspension monitoring. Raman spectra were collected at a laser intensity of 150 mW in the \(-50 – 3600 \text{ cm}^{-1}\) range with a resolution of 0.5 cm\(^{-1}\) and were averaged over 10 scans using an exposure time of 5 seconds.

### 2.2.3 FBRM

The focused beam reflectance measurement (FBRM) allows for in situ measurements of the chord length distribution (CLD). From the obtained CLD, the actual particle size distribution can in principle be restored\(^{26-30}\). The measurement principle of the FBRM is illustrated in Figure 2.4. A laser light source produces a continuous beam of monochromatic light that is focused to a small spot using a set of lenses. A pneumatic or electrical motor is used to rotate the optics, such that a rotating, focused beam of laser light is sent into the suspension to be analyzed. Particles in the suspension will backscatter the laser light to the probe where it is detected. From the duration of the backscatter and the rotation velocity of the optics, the distance the beam has traveled over the particle surface, the so called chord length, can be calculated.
2.2 In situ techniques

Figure 2.4: The measurement principle of the FBRM. Particles in suspension backscatter the laser light emitted by the probe. The distance the light travels over the particle surface is the chord length.

In this work, a laboratory scale FBRM 600L from Lasentec (Redmond, WA, USA) has been applied to detect the onset of particle formation during the ATR-FTIR calibration measurements and to verify that no significant nucleation occurred during seeded desupersaturation experiments.

2.2.4 In situ imaging

Another method to measure particle size distributions in situ is through imaging techniques. A variety of in situ imaging techniques are available, among them the Particle Vision and Measurement (PVM) instrument. A drawback is the fact that these techniques are effective only in the case suspension density is relatively low. In case of a higher suspension density, particles in suspension overlap on the images and automated image analysis routines are likely to provide incorrect particle size distributions.
2. Experimental

2.3 Ex situ techniques

Not all properties of crystals can be measured in situ, hence several ex situ characterization techniques were used in this work. However, these measurements required sampling and subsequently filtration and drying steps.

2.3.1 Powder X-ray diffraction

X-ray diffraction is a method to determine the arrangement of atoms within a crystal structure. A beam of X-rays strikes a crystal and scatters into many different directions; from the angles and intensities of these scattered beams, the three dimensional structure of the crystal lattice can be elucidated. In this work, powder X-ray diffraction was applied mainly to distinguish between different crystal forms of the same substance, i.e. to distinguish different polymorphs, as an additional technique next to Raman spectroscopy.

Powder X-ray diffraction patterns were recorded after samples were spread uniformly over the samples holder using a AXS D8 Advance diffractometer from Bruker (40 kV, 40 mA, Cu K\text{α}) (Karlsruhe, Germany). Patterns were recorded at $2\theta = 2 - 45^\circ$ with a step size of 0.02° and a scan speed of 0.4°/min.

2.3.2 Multisizer

The Multisizer employs the change in the electrical impedance that occurs when a particle flows through an aperture placed between two electrodes to measure particle size distributions.
2.3 Ex situ techniques

Particle size distributions (PSDs) were measured using a Multisizer 3 from Beckman Coulter (Nyon, Switzerland). This device uses the Coulter Principle to measure the number and volume particle size distribution with a high resolution in an overall range of 0.4 – 1200 µm. Every measurement consisted of at least 40,000 particles and PSDs were smoothed with a moving average filter. Measurements were performed by adding approximately 100 mg of crystals to 200 ml of a saturated solution at room temperature. Free ions are required for this measurement technique and in the case these were not present, 2 wt.% NaCl was added.

2.3.3 Microscopy

Scanning electron microscopy (SEM) samples were sputtered with approximately 2 nm of platinum in high vacuum before being recorded with a Leo 1530 microscope from Zeiss/LEO (Oberkochen, Germany). Optical microscopy using a Zeiss Axioplan (Feldbach, Switzerland) was employed to quickly assess qualitative information about particle size and shape of crystals.
Chapter 3

Modeling

In this chapter, the theoretical background will be discussed that forms the basis for the results presented in this work. In Section 3.1, the analysis of time-resolved spectroscopic data through a multivariate approach is presented. This section discusses classical calibration-based methods as well as novel calibration-free methods. The mathematical description of crystallization processes is presented in Section 3.2. Finally, the parameter estimation techniques that were used throughout this work, will be addressed briefly in Section 3.3.

3.1 Modeling spectroscopic data

The underlying assumption for the analysis of spectroscopic data is a linear dependency of the measured spectra on the concentration of the involved components or analytes. This dependency is known in the case
of infrared spectroscopy as the Beer-Lambert law, as discussed in Section 2.2.1, and in the case of Raman spectroscopy, the scattering intensity depends linearly on the amount of scattering material per unit volume, which can be present both in the solution and in the solid phase in the case of suspensions. On this basis, the analysis of spectroscopic data presented below is the same for ATR-FTIR and for Raman spectroscopy. Assuming this linear dependency, the measured signal intensity at a certain Raman shift or wavenumber $\lambda$ and at a given time $t$, $x(t, \lambda)$, can then be expressed as a linear combination of the signals corresponding to each of the $d$ analytes, i.e.:

$$x(t, \lambda) = \sum_{l=1}^{d} c_l(t) a_{l}(\lambda) + e(t, \lambda) = \hat{x}(t, \lambda) + e(t, \lambda) \quad (3.1)$$

where $a_{l}(\lambda)$ represents the intensity at Raman shift or wavenumber, $\lambda$, of the pure-analyte spectrum corresponding to the $l^{th}$ analyte, $c_l(t)$ denotes the concentration of the $l^{th}$ analyte at time $t$, and $e(t, \lambda)$ represents the experimental error, i.e. the noise and the nonidealities of the measurement. Taking the discretized spectral coordinate, the course of the $j^{th}$ Raman shift or wavenumber over time can therefore be expressed as:

$$x_{j}(t) = \sum_{l=1}^{d} c_l(t) a_{l,j} + e_{j}(t) \quad (j = 1, ..., m) \quad (3.2)$$

where the index $j$ corresponds to a specific Raman shift or wavenumber $\lambda_{j}$. By sampling the time coordinate at $t_1$, $t_2$, ..., $t_i$, ..., $t_n$, the $i^{th}$ spectral signal at the $j^{th}$ Raman shift or wavenumber can finally be
written as:

\[ x_{i,j} = \sum_{l=1}^{d} c_{i,l} a_{l,j} + e_{i,j} \quad (i = 1, \ldots, n), \ (j = 1, \ldots, m), \quad (3.3) \]

which can be recast in matrix notation as:

\[ X = CA + E = \hat{X} + E \quad (3.4) \]

where \( X \) \((n \times m)\) is the spectral matrix in which element \( x_{ij} \) represents the \( j^{th} \) measured intensity for the \( i^{th} \) sample and \( C \) \((n \times d)\) represents the state matrix, i.e. the concentrations of the \( d \) analytes involved, where the \( l^{th} \) column of \( C \) is the concentration profile in time of the \( l^{th} \) analyte. The \( l^{th} \) row in the matrix \( A \) \((d \times m)\) denotes the discretized pure-analyte spectrum for analyte \( l \); the matrix \( E \) \((n \times m)\) is the matrix of experimental errors. It is worth noting that the discretization of the Raman shift or wavenumber coordinate as well as the sampling in time are determined by the settings of the instruments, i.e. the spectrometers.

### 3.1.1 Calibration-based approaches

One approach to apply spectroscopy in a quantitative manner is to assume that the state matrix \( C \) can be obtained from the measured spectral matrix \( X \) as:

\[ C = XB + E \quad (3.5) \]

where \( B \) \((m \times d)\) is the regression matrix and \( E \) \((n \times d)\) denoted the experimental error. If the matrix \( A \) given in eq. (3.4), which is in general not square since \( d \neq m \), were known then the regression matrix
3.1 Modeling spectroscopic data

B could be calculated as

$$B = A^T(AA^T)^{-1},$$

(3.6)

which is obtained by neglecting the experimental error, i.e. $E = 0$, and multiplying both sides of eq. (3.4) on the right by $A^T$. However, the matrix of pure-analyte spectra $A$ is normally not known. Then, the approach to determine $B$ is to perform a series of ad hoc calibration measurements with known concentrations $C_{cal}$, thus resulting in a spectral matrix $X^{cal}$. The regression matrix $B$ is then calculated by minimizing in some sense the difference between $C^{cal}$ and $X^{cal}B$. This approach works, but requires a time-consuming calibration procedure. It is the only approach when the interest is to use spectroscopy to measure the concentrations of analytes unrelated to the time-evolution of a process or to a process model.

Typically, a multiple linear regression (MLR) approach might apply and the regression matrix $B$ can be found through a least-squares problem formulated as

$$\min_B \|C^{cal} - X^{cal}B\|$$

(3.7)

where $\| \cdot \|$ denotes the Euclidean norm. In the case of a square spectral matrix $X^{cal}$, i.e. $n = m$, $B$ can be found through inversion of $X^{cal}$, i.e. $B = X^{cal-1}C^{cal}$. However, $X^{cal}$ is usually not square and the solution of eq. (3.7) is given by

$$B = (X^{calT}X^{cal})^{-1}X^{calT}C^{cal}.$$  

(3.8)

In spectroscopic studies, the number of variables $m$ is generally very large and the variables are correlated, hence the matrix $X^{cal}$ is ill-conditioned and the approach given by eq. (3.8) cannot be employed.
Several approaches can be applied to deal with regression problems for ill-conditioned matrices. In this work, established projection based methods such as principal component regression (PCR) and partial least squares regression (PLSR) are employed\textsuperscript{32,33}.

**Projection based methods**

In the following only one analyte, and therefore only one concentration profile, is considered. Therefore, the concentration matrix $\mathbf{C}$ reduces to column vector $\mathbf{c}$. It should be noted that all approaches also work for more than one analyte, however their derivations are more complex and can be found elsewhere\textsuperscript{34}. For the sake of clarity, the superscript $\text{cal}$ in the spectral matrix has been left out.

In both PCR and PLSR, the matrix $\mathbf{X}$ is projected to a scores matrix $\mathbf{T}_r$ ($n \times r$), $r$ being a small number of linear combinations of the original variables that still carry the relevant information about $\mathbf{X}$. Typically, the dimension of the regression problem is reduced from $m$ ($10^2$ to $10^4$) strongly correlated or even linearly dependent variables to $r$, i.e. 1 to 10, linearly independent variables that are linear combinations of the original variables, therefore the regression model can be written as

$$\mathbf{c} = \mathbf{T}_r \mathbf{b}_r + \mathbf{e}_r$$  \hspace{1cm} (3.9)

with $\mathbf{b}_r$ ($r \times 1$) and $\mathbf{e}_r$ ($n \times 1$), whose exact least squares solution is

$$\mathbf{b}_r = (\mathbf{T}_r^T \mathbf{T}_r)^{-1} \mathbf{T}_r^T \mathbf{c}.$$  \hspace{1cm} (3.10)

It should be emphasized that this approach has the important advantage that $\mathbf{T}_r$ is by construction not ill-conditioned and the regression vector $\mathbf{b}_r$ is small. PCR and PLSR differ in how the matrix $\mathbf{T}$ of linear
3.1 Modeling spectroscopic data

combinations of the original variables, the scores, is obtained. For both PCR and PLSR the general expression defining the scores is

$$T = XW$$  \hspace{1cm} (3.11)

where $W$ ($m \times m$) is the matrix of weights, i.e. the $j^{th}$ column of $W$ is the vector of weights that defines the $j^{th}$ column in $T$ ($n \times m$), i.e. the $j^{th}$ new variable.

**Principal component regression**

Starting point of principal component regression (PCR) is principal component analysis (PCA). In PCA the vector $w_j$ ($j^{th}$ column of $W$) is chosen in such a way that the elements of $t_j$ ($j^{th}$ column of $T$) discriminate most among the measured samples, i.e. the variance of $t_j$ is maximized. Standard linear algebra\textsuperscript{34,35} demonstrates that this corresponds to taking as $w_j$ the eigenvector of the matrix $X^TX$; the variance of $t_j$ is therefore given by the $j^{th}$ eigenvalue $\lambda_j$ of the matrix $X^TX$ (which equals the square of the singular value $s_j$ of matrix $X$). The approach is to chose the eigenvectors according to the decreasing order of their corresponding eigenvalues, i.e. $\lambda_1 \geq \lambda_2 \geq ... \geq \lambda_m$. Thus, the variance of the vectors $t_1, t_2, ..., t_j, ...$, is decreasing, hence the linear combinations of original variables, $t_j$, for increasing $j$, account for less and less variability in the original data. Therefore, only a small number $r$ of vectors $t_j$ is sufficient, thus resulting in the matrix $T_r$ ($n \times r$), which contains only the first $r$ columns of $T$. This approach can also be explained by employing the technique of singular value decomposition (SVD) on matrix $X$. The regression vector $b_r$ is given by eq. (3.10).

The concentration profile $\tilde{c}$ ($k \times 1$) of a certain series of measurements, whose spectra are stored in the matrix $\tilde{X}$ ($k \times m$), can then be estimated
as

$$\tilde{c} = \tilde{T}_r b_r + \tilde{e}_r$$  \hspace{1cm} (3.12)

where the matrix $\tilde{T}_r (k \times r)$ can be found through projection of $\tilde{X}$ using the weights $W_r (m \times r)$

$$\tilde{T}_r = \tilde{X}W_r.$$  \hspace{1cm} (3.13)

Many examples can be found in literature that use a different notation for the weights $W$, namely loadings $P$ that regress $X$ on $T$.

**Partial least squares regression**

It should be emphasized that in partial least squares regression (PLSR), the same symbols are used for the scores, weights and loadings as in PCR. However, the scores, weights and loadings are estimated differently. In PLSR the vector $w_j$ is chosen in such a way that the capacity of $t_j$ to describe $c$, i.e. to account for the differences of the elements in $c$ corresponding to the different measurements, is maximized. This corresponds to maximizing the covariance between $t_j$ and $y$. Using standard linear algebra$^{34}$ it can be shown that

$$w_1 = \frac{X^T c}{\|X^T c\|}$$  \hspace{1cm} (3.14)

where $w_1$ represents the first column vector of weights. After finding the first column $t_1 = Xw_1$ of the matrix $T$, $w_2$ is calculated in the same way from a deflated form of the matrix $X$, and so on and so forth. There are two different ways to deflate the matrix $X$ resulting in different loadings $P$ and details can be found elsewhere$^{34}$. The overall expression of PLSR can be written as

$$X = T_r P_r^T + E_r.$$  \hspace{1cm} (3.15)
3.1 Modeling spectroscopic data

\[ c = T_r b_r + e_r \]  \hspace{1cm} (3.16)

where \( T_r \) \((n \times r)\) is a matrix of scores, \( P_r \) \((m \times r)\) is a matrix of loadings and the column vector \( b_r \) \((r \times 1)\) represents the regression vector. Matrix \( E_r \) \((n \times m)\) and column vector \( e_r \) \((n \times 1)\) are the residual matrix and the residual column vector, respectively. Regression vector \( b_r \) can again be found by applying eq. (3.10).

As in PCR, eq. (3.12) and eq. (3.13) can be used to estimate the unknown concentrations of a series of measurements using the constructed linear combinations of original variables.

**General features**

Both PCR and PLSR concern ill-conditioned regression problems. Although the overall expressions for PCR and PLSR are similar, the estimated scores and loadings are different. In general, PCR and PLSR are not expected to yield the same solution, however, solutions are expected to be similar\(^{34}\). The optimal number of principal components (PCs) in case of PCR or latent variables (LVs) in case of PLSR is generally found through a cross-validation procedure. During cross-validation, parts of the calibration set are left out of the calculations and kept separately, the model is build using a certain number of PCs or LVs, and applied to estimate the subset of samples that was left out. This procedure is repeated several times for different subsets and the root-mean-square of the squared differences (RMSECV, root-mean-square error of cross-validation) between estimated and real values is employed as a selection criterion for the number of principal components or latent variables. The optimal number of principal components or latent variables is found where the RMSECV as a function of the number of principal components or latent variables reaches a minimum. There are various
cross-validation methods that differ in how the samples are left out of the calibration set. Two methods have been applied in this work, i.e. leave-one-out and random-subset cross-validation\textsuperscript{36}. Both methods gave very similar results, in all cases the RMSECV for leave-one-out was found to be slightly lower as compared to random-subset RMSECV. In this work, all reported values for the RMSECV result from the leave-one-out cross-validation approach.

**Preprocessing techniques**

Before construction of a multivariate model, spectra can be processed in order to remove unwanted variation in the data, e.g. shifting baselines in the spectra, hence improving model performance. Preprocessing techniques range from centering of the data, smoothing, baseline corrections to taking derivatives. A variety of preprocessing methods was studied in this work, among them are linear baseline correction, spectral normalization, standard normal variate transformation (SNV) and multiplicative scatter correction (MSC). In the following, the latter two preprocessing techniques are briefly presented.

In both the SNV and MSC approaches, one subtracts an offset \( a_i \) and divides the resulting spectrum by a scaling factor \( b_i \), i.e. one applies the following formula:

\[
x_{ij}^{pp} = \frac{x_{ij} - a_i}{b_i}
\]  

(3.17)

where \( x_{ij}^{pp} \) represents the \( j^{th} \) element of the \( i^{th} \) preprocessed spectrum. The SNV approach centers each spectrum around its mean and scales it to its standard deviation, resulting in the following expressions for \( a_i \)
3.1 Modeling spectroscopic data

and \( b_i \):\(^{37}\)

\[
\begin{align*}
a_i^{snv} &= \bar{x}_i = \frac{\sum_{j=1}^{m} x_{ij}}{m} \\
b_i^{snv} &= \sqrt{\frac{\sum_{j=1}^{m} (x_{ij} - \bar{x}_i)^2}{m - 1}}. 
\end{align*}
\] (3.18)

The MSC method is similar, however, it uses the information of the entire spectral matrix to treat the individual spectra. It is assumed that the observed spectrum is the result of an offset, a multiplicative factor and a residual:

\[
x_{ij} = a_i + b_i \bar{x}_j + s_{ij}
\] (3.19)

where \( a_i \) and \( b_i \) are estimated by ordinary least squares regression of the \( i^{th} \) spectrum against the average spectrum \( \bar{x} \) (1 \( \times \) \( m \)):

\[
\begin{align*}
a_i^{msc} &= \frac{\sum_{j=1}^{m} x_{ij}}{m} - b_i^{msc} \frac{\sum_{j=1}^{m} \bar{x}_j}{m} \\
b_i^{msc} &= \frac{\sum_{j=1}^{m} x_{ij} \bar{x}_j - (\sum_{j=1}^{m} x_{ij})(\sum_{j=1}^{m} \bar{x}_j)/m}{\sum_{j=1}^{m} \bar{x}_j^2 - (\sum_{j=1}^{m} \bar{x}_j)^2/m}.
\end{align*}
\] (3.20)

Spectrum \( \bar{x} \) is a reference spectrum, usually the average spectrum of the calibration set. In eq (3.19), \( s_{ij} \) represents the residual of element \( x_{ij} \) and the spectrum \( s_i \) represents the relevant information in \( x_i \).\(^{38}\)

All computations were performed using MATLAB 7.1 and PLS_Toolbox 4.0 developed by Eigenvector Research Inc. (Manson, WA, USA).
3.1.2 Calibration-free approaches

Let us assume that the process considered in Section 3.1 can be described by a model that calculates how the concentrations of the different analytes evolve in time. Let us call the calculated concentrations \( \hat{c}_l \), which can be expressed as:

\[
\hat{c}_l = \hat{c}_l(t, k) \tag{3.21}
\]

where \( k \) is the set of \( p \) parameters that appear in the model equations, e.g. physical properties of the system. As in Section 3.1, one can construct the time-resolved concentration matrix \( \hat{C}(k) \). Using the calibration-based approach presented in Section 3.1.1, i.e. calculating the concentration matrix \( C = XB \) from the measured spectral matrix \( X \), one can estimate the model parameters \( k \) by minimizing in some sense the difference between the matrices \( \hat{C}(k) \) and \( C \). This is the classical approach that relies on a, in most cases, lengthy calibration procedure.

Let us assume on the contrary that no calibration set is available, i.e. neither the matrix of pure-analyte spectra \( A \) nor the regression matrix \( B \) are known. The idea on which the proposed method is based is that of using the time-resolved Raman spectra, i.e. the matrix \( X \) itself, for the estimation of the model parameters. Formally, this would imply minimizing for instance the sum \( S_r \) of the squares of the elements of the residual matrix \( R(k) \) \((n \times m)\):

\[
R(k) = X - \hat{C}(k)A \tag{3.22}
\]

where both the \( p \) elements of the vector \( k \) and the \( d \times m \) elements of the matrix \( A \) are unknown and should be obtained through some optimization procedure, i.e. a rather formidable task considering the number of elements of \( A \) can easily be several hundreds or even thousands.
However, assuming eq. (3.22) can be solved exactly, one can write the following sequence of equations:

\[ X = \hat{C}(k)A \]  
\[ \hat{C}^T(k)X = \hat{C}^T(k)\hat{C}(k)A \]  
\[ A = (\hat{C}^T(k)\hat{C}(k))^{-1}\hat{C}^T(k)X = \hat{C}^+(k)X. \]  

Substituting the last equation into eq. (3.22) yields:

\[ R(k) = X - \hat{C}(k)\hat{C}^+(k)X = [I - \hat{C}(k)\hat{C}^+(k)]X. \]

By minimizing the sum of the squares of the elements of the residual matrix \( R(k) \), one obtains the \( p \) unknown model parameters, i.e. a much easier task than solving eq. (3.22)\textsuperscript{39–41}.

A couple of remarks are worth making. First, the matrix \( \hat{C}^+(k) \), which is defined by eq. (3.25), can be obtained more efficiently through singular value decomposition (SVD),\textsuperscript{35} i.e. by writing \( \hat{C}(k) = USV^T \) and \( \hat{C}^+(k) = VS^{-1}U^T \), where \( U \ (n \times d) \), \( S \ (d \times d) \) and \( V \ (d \times d) \) with \( U^TU = I \), \( V^TV = VV^T = I \) and \( S \) is a diagonal matrix with the singular values of \( \hat{C}(k) \) in decreasing order on its diagonal. Second, once the model parameters \( k \) are estimated, one can in principle use \( X \) and \( \hat{C}(k) \), whose elements are now known, to calculate a regression matrix for other purposes, following the procedure as described in Section 3.1.1.

The technique proposed should not be confused with multivariate curve resolution (MCR), a technique that estimates the matrices \( C \) and \( A \) entirely through optimization without a process model but using only physical restrictions, e.g. concentration can not be negative and fulfillment of the mass balance\textsuperscript{40,42,43}. 

27
3.2 Process modeling

3.2.1 Population balance equation

Population balances are used in several branches of modern science, mainly concerning particulate processes such as crystallization, aerosol engineering and polymer technology. A population balance equation (PBE) defines how a population of separate entities develops over time. Therefore, PBEs belong to a subcategory of equations known as partial differential equations. The formulation of population balances in the case of crystallization is based on the number density of crystals, i.e. \( n(t, L) \). The population balance equation results from examining the number of particles in a differential crystal size interval \( dL \). In the case of a perfectly mixed batch crystallizer with constant suspension volume and assuming size-independent growth and neither agglomeration nor breakage, the PBE reads as

\[
\frac{\partial n}{\partial t} + G \frac{\partial n}{\partial L} = 0 \tag{3.27}
\]

where \( L \) is the particle size, \( t \) is the time, \( n \) is the number density of crystals, i.e. its particle size distribution (PSD), and \( G \) denotes the crystal growth rate. The solute material balance, which defines the solute concentration, \( c(t) \), is written as:

\[
\frac{dc}{dt} = -3k_v \rho G \int_0^\infty L^2 n(t, L) dL \tag{3.28}
\]

where \( k_v \) and \( \rho \) denote the volume shape factor and the crystal density, respectively. The following initial and boundary conditions apply to the
3.2 Process modeling

PBE and to the material balance equation:

\[ n(0, L) = n_0(L) \quad (3.29) \]
\[ n(t, 0) = \frac{J}{G} \quad (3.30) \]
\[ c(0) = c_0 \quad (3.31) \]

where \( J \) is the nucleation rate and \( c_0 \) and \( n_0(L) \) denote the initial solute concentration and the initial particle size distribution, respectively. The supersaturation, \( S(t) \), required to calculate the crystal growth and nucleation rates, is defined as:

\[ S = \frac{c}{c^*} \quad (3.32) \]

with \( c^* \) being the solubility.

### 3.2.2 Solution of the population balance equation

Often, the population balance equation cannot be solved analytically and therefore numerical solutions are needed. Several approaches exist to solve population balance equations, each having its own advantages and disadvantages depending on the considered process and corresponding phenomena.

**Method of Kumar and Ramkrishna**

The moving pivot technique proposed by Kumar and Ramkrishna\(^{44} \) has proven to be robust and reliable for many different processes. Following this approach, the PBE is discretized and each size range is represented by a corresponding length, the pivot, and its lower and upper boundary.
In most cases, the arithmetic mean of the size of the lower and upper boundaries is used as the size of the pivot. The moving pivot technique overcomes the problem of numerical diffusion and instability, while providing the needed accuracy and requiring a reasonable computational effort.\(^\text{44}\) The discretized form of eq. (3.27) is

\[
\frac{dN_j}{dt} = J\delta_{j,1} \tag{3.33}
\]

\[
\frac{dx_j}{dt} = G \quad (j > 1) \tag{3.34}
\]

\[
\frac{dx_1}{dt} = \frac{1}{2}G \tag{3.35}
\]

where \(N_j\) represents the total number of particles in the \(j^{\text{th}}\) size range, \(\delta_{j,1}\) represents the Kronecker delta and \(x_j\) is the size of the \(j^{\text{th}}\) pivot. Essentially, the right-hand-side of eq. (3.33) reduces to zero for all size ranges except the smallest one. Eqs. (3.33), (3.34) and (3.35) form a system of coupled ordinary differential equations and can be solved using for instance the MATLAB \textit{ode45} solver.

**Method of moments**

Alternatively, the method of moments\(^\text{45}\) can be applied to obtain a numerical solution of eq. (3.27). The \(j^{\text{th}}\) moment of the PSD of a population of crystals is defined as

\[
\mu_j(t) = \int_0^\infty L^j n(t, L) dL. \tag{3.36}
\]

In case no nucleation occurs, the time derivative of the zeroth moment equals zero:

\[
\frac{d\mu_0}{dt} = 0; \tag{3.37}
\]
whereas the equations for all higher moments are:

\[
\frac{d\mu_j}{dt} = jG\mu_{j-1} \quad (j \geq 1). \tag{3.38}
\]

Using the second and third moment, the solute mass balance eq. (3.28) can be recast as:

\[
\frac{dc}{dt} = -3k_v \rho G \mu_2 = -k_v \rho \frac{d\mu_3}{dt}. \tag{3.39}
\]

The solid concentration of crystals can be calculated using the third moment:

\[
m(t) = k_v \rho \mu_3(t), \tag{3.40}
\]

and by combining eq. (3.39) with eq. (3.40) one obtains

\[
c(t) = c_0 + m_0 - m(t), \tag{3.41}
\]

where \(m_0\) denotes the mass of seed crystals, i.e. \(m(0)\).

Eqs. (3.37) and (3.38), for \(j = 1, 2, 3\), form a system of four coupled ordinary differential equations combined with the mass balance eq. (3.41), which can be solved once the initial values of the four moments of the PSD and of the solute concentration are fixed. The \texttt{ode45}, \texttt{ode15s} and \texttt{ode23} solvers in the MATLAB programming environment were found to be robust and fast in solving this problem.

In the case nucleation takes place, the time derivative of the zeroth moment equals the nucleation rate:

\[
\frac{d\mu_0}{dt} = J. \tag{3.42}
\]

In the case a crystal form nucleates, grows and subsequently dissolves,
i.e. in the case of a solvent-mediated polymorph transformation, the time-derivative of the zeroth moment depends on the value of the supersaturation:\textsuperscript{46,47}

\[
\frac{d\mu_0}{dt} = \begin{cases} 
  Dn_t^\ast(L^\ast), & \text{if } S \leq 1 \\
  J, & \text{if } S > 1 
\end{cases} \quad (3.43)
\]

where \(D\) represents the dissolution rate in the case that \(S \leq 1\) and the characteristic length \(L^\ast\) can be calculated as:

\[
L^\ast = \left| \int_{t^\ast}^t D dt \right|. \quad (3.44)
\]

In case of a constant dissolution rate, \(L^\ast\) equals \(|D(t - t^\ast)|\), however in case of a non-constant dissolution rate the integral of eq. (3.44) has to be calculated numerically. In eqs. (3.43) and (3.44), \(t^\ast\) represents the time where the dissolution starts, i.e.

\[
t^\ast = \min\{t(S \leq 1)\}. \quad (3.45)
\]

For a seeded polymorph transformation \(t^\ast\) equals 0 and \(n_t^\ast\) represents \(n_0\), i.e. the particle size distribution of the seed crystals; however, in the case of an unseeded polymorph transformation, the meta-stable form nucleates and grows initially, and, after the supersaturation is consumed, it dissolves. At the point in time where the dissolution of the metastable form starts, i.e. the point where \(S_\alpha \leq 1\) for the first time, the PSD has to be estimated in order to apply eq. (3.43). From this particular point in time, i.e. \(t^\ast\), an unseeded transformation evolves the same as a seeded polymorph transformation.

One way to reconstruct the particle size distribution is through fitting splines (piecewise polynomials) using a finite number of moments; the details of this method can be found elsewhere\textsuperscript{48,49}. 
A second and more elegant way to reconstruct a particle size distribution in the case nucleation and growth both take place is the recently proposed Laplace transform technique\textsuperscript{45}. Given that the values of the nucleation and growth rates over time are available, e.g. obtained through the method of moments, the PSD at a given time $t$ can be found as follows:

$$n(t, L) = n_0 \left( L - \int_0^t G(\tau)d\tau \right) + r(L) \quad (3.46)$$

where $r(L)$ is defined as:

$$r(L) = \begin{cases} \frac{J(\xi)}{G(\xi)}, & \text{if } (L - L_0) \in (0, u(0, t)] \\ 0, & \text{otherwise} \end{cases} \quad (3.47)$$

where $u(0, t)$ equals

$$u(0, t) = \int_0^t G(\tau)d\tau. \quad (3.48)$$

To use eq. (3.47), $\xi$ has to be estimated numerically by finding the root of the implicit function $F(\xi)$ defined as

$$F(\xi) = \int_{\xi}^t G(\tau)d\tau - (L - L_0). \quad (3.49)$$

In this work, the root of eq. (3.49) was determined using Newton’s formula\textsuperscript{35}, which was found to give accurate and robust results using a short computation time.

In the case of pure crystal growth, i.e. nucleation is not taken into account, $r(L)$ in eq. (3.46) equals zero and the reconstructed PSD equals the PSD of the seed crystals shifted towards larger particle sizes, a result that could also be obtained analytically through the method of characteristics\textsuperscript{50}. 
3. Modeling

3.2.3 Constitutive equations

Combined with proper nucleation, growth and dissolution rate expressions, a population balance equation based model allows for the calculation of the solute and solid concentrations and the particle size distribution over time. Several approaches exist to express the nucleation, growth and dissolution rates as a function of the process variables, e.g. the supersaturation, temperature or suspension density. These expressions are often first-principle expressions based on a specific mechanism, e.g. the birth-and-spread and screw dislocation mechanisms\textsuperscript{1,51}. In this work however, the objective was not to reveal these mechanisms but to obtain a descriptive process model that could be used to predict the evolution of the solute and solid concentrations and the particle size distribution over time. For this reason, relatively simple empirical expressions have been used in most cases to describe the nucleation, growth and dissolution kinetics.

Nucleation kinetics

Nucleation phenomena, i.e. the formation of crystals from a liquid phase, can be divided into primary and secondary nucleation phenomena. Primary nucleation takes place in the absence of crystals and can be subdivided into homogeneous, in the absence of any foreign particles such as dust, and heterogeneous, in the presence of facilitating foreign particles, nucleation. The primary nucleation rate $J_{pri}$, i.e. the number of nuclei formed per second per unit volume, can be expressed as follows:

$$J_{pri} = J_{hom} + J_{het} = k_{J_1} \exp \left( - \frac{k_{J_2}}{\ln^2 S} \right) + k_{J_3} \exp \left( - \frac{k_{J_4}}{\ln^2 S} \right).$$  \hspace{1cm} (3.50)
3.2 Process modeling

In this work, relatively low supersaturation values have been applied, hence the homogeneous part could always be neglected.

Secondary nucleation occurs when crystals are already present and can result from effects such as shear, breakage and abrasion. A lumped expression for the secondary nucleation rate was proposed in the literature\(^1\):

\[
J_{sec} \sim f_1(\text{mechanisms}) f_2(S) \varphi^i \bar{\epsilon}^j
\]  

(3.51)

where \(f_1\) is the number of attrition fragments formed by collisions, \(f_2\) is the fraction of particles that grow and survive, \(\varphi\) is the volumetric crystal holdup, and \(\bar{\epsilon}\) is the mean specific power input. The mean specific power input \(\bar{\epsilon}\) can be estimated using a power number correlation

\[
\bar{\epsilon} = \frac{N_p d_{imp}^5 n_s^3}{V}
\]  

(3.52)

where \(N_p\) is the power number for the stirrer type used, \(d_{imp}\) is the impeller diameter, \(n_s\) is the stirring rate, and \(V\) is the reactor volume.

**Growth kinetics**

Crystal growth 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. In this work, only integration controlled crystal growth will be considered. Generally, the linear growth rate \(G\) is defined as the change of a characteristic crystal dimension per unit time:

\[
G = \frac{dL}{dt}
\]  

(3.53)
where $L$ denotes the characteristic length of the crystal and $t$ is the time. Two of the most widely used integration controlled crystal growth mechanisms are the birth-and-spread and the screw dislocation mechanisms. In this work, the birth-and-spread mechanism was found to be adequate for all systems that were studied and its resulting crystal growth rate at a specific temperature can be expressed as

$$G = k_{G1}(S - 1)^{2/3}(\ln S)^{1/6} \exp \left( -\frac{k_{G2}}{\ln S} \right). \quad (3.54)$$

### Dissolution kinetics

The dissolution of crystals takes place when the supersaturation value is lower than unity, i.e. the solute concentration is lower than the solubility of the crystalline form. Dissolution of crystals is often assumed to be limited by mass transfer effects and therefore its rate can be estimated as follows:

$$D = k_D(S - 1) \quad (3.55)$$

where $k_D$ represents the mass transfer coefficient that could in principle be estimated using a Sherwood correlation$^{1,52}$. 

### 3.2.4 Illustrative example

To illustrate the evolution of the solute and solid concentrations and the particle size distribution over time, a seeded batch desupersaturation experiment during which nucleation takes place will be discussed briefly. During a seeded batch desupersaturation experiment, an amount of previously characterized seeds crystals is added to a supersaturated solution at constant temperature. In this particular example, it was assumed nucleation takes place simultaneously to crystal growth. Figure 3.1 il-
3.2 Process modeling

Figure 3.1: The time-evolution of the first four moments (a) and the concentration (b) and the resulting particle size distribution at the end of the process (c).

Illustrates the evolution of this crystallization process during which nucleation and crystal growth take place simultaneously. Figure 3.1(a) shows the evolution of the first four moments over time. To facilitate comparison of the individual moments, their values have been normalized with their initial values. Figure 3.1(b) displays the solute concentration over time, which is obtained from the material balance eq. (3.41). The concentration of solid is determined from the moments. The initial and final particle size distribution can be seen Figure 3.1(c). As it can be seen, the initial distribution is indeed shifted towards larger particle sizes due
to crystal growth. The part on the left is due to nucleation.

### 3.3 Parameter estimation

In this work, kinetic parameters have been estimated using a variety of optimization algorithms. All algorithms were readily available in the MATLAB programming environment and used without further modifications. Frequently used methods such as the simplex method and the Newton-Gauss-Levenberg/Marquardt (NGL/M) approach were applied using the `fminsearch` and `lsqnonlin` functions in MATLAB, respectively\(^3\). Additionally, simulated annealing, an approach that is known to be effective for elucidating a global minimum in the presence of several local minima, was used by employing the `simulannealbnd` function in MATLAB\(^3,5\).

Once the unknown parameters are estimated, their confidence intervals represent their sensitivity, i.e. the smaller the confidence interval the smaller the sensitivity of the parameter\(^3,5\). Approximate confidence intervals can be calculated using the sensitivity matrix or Jacobian based on a linearized model in the vicinity of the estimated model parameters. This sensitivity matrix enables calculating the covariance matrix of the parameter estimates. The standard error \(s_k\) of the \(k^{th}\) parameter \(k_k\) is given by the square root of the \(k^{th}\) diagonal element of this covariance matrix. The confidence interval of the \(k^{th}\) parameter is given by

\[
    k_k \pm t_{\alpha, \nu} s_k, \tag{3.56}
\]

where \(t_{\alpha, \nu}\) is the value of the t-distribution for \(\nu\) degrees of freedom, i.e. the number of data points minus the number of parameters, and confidence level \(\alpha\). Here \(\alpha = 0.05\) was used, resulting in a 95% probability.
Chapter 4

Quantitative application of in situ ATR-FTIR and Raman spectroscopy

In this chapter, different process analytical technologies (PATs) based on vibrational spectroscopy, i.e. attenuated total reflectance Fourier transform infrared (ATR-FTIR) and Raman spectroscopy, are applied by means of multivariate data analysis techniques. Wide applicability has been demonstrated by in situ monitoring of various crystallization processes, e.g. solubility curve measurement, cooling crystallization and solvent-mediated polymorph transformation. A calibration strategy has been proposed to obtain accurate and robust estimations of


39
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

the solute concentration by ATR-FTIR monitoring. Different calibration models and preprocessing techniques were applied and compared. It was shown that these methods allow for solute concentration monitoring of nonisothermal processes even for sparingly soluble substances such as L-glutamic acid in an aqueous environment. An extensive study has been performed to identify the underlying process parameters that influence the Raman signal. It is demonstrated that principal component analysis provides qualitative information for seeded and unseeded polymorphic transformations and enables endpoint determination of a solid-state transformation process using L-glutamic acid. The multivariate calibration approach described in this work allows for quantitative application of Raman spectroscopy on a multiphase multicomponent dynamic process such as a solvent-mediated polymorphic transformation. Additionally, it was shown that multivariate analysis of Raman data allows for solute concentration estimation despite the fact that the solute signals are weak and completely overlapping with the signals related to the solid phase.

4.1 Introduction

A thorough and in-depth understanding of the physical and chemical phenomena involved in pharmaceutical and fine-chemical unit operations is of increasing importance. To this aim, reliable and robust real-time information about a given process is essential since it contributes significantly to process understanding and is a requirement for process control purposes. However, one deals mostly with multicomponent multiphase processes, such as crystallization and precipitation for which obtaining reliable and robust real-time information is challenging.\textsuperscript{55–59}
Besides particle size and shape, solid composition is crucial in understanding (pseudo-)polymorphism, which is of key importance in the pharmaceutical and fine-chemical industry since different (pseudo-) polymorphs have different physical and chemical properties, such as solubility and reactivity. The solid phase composition can be assessed in situ through various characterization techniques, i.e. powder X-ray diffraction\(^{31,60}\), near-infrared\(^{61,62}\) and Raman spectroscopy\(^{7,63–66}\). Raman spectroscopy has received significant attention as a potential technique to characterize pharmaceuticals in powder mixtures as well as in suspensions\(^7\). However, the estimation of the solid composition is known to be challenging since several process variables, i.e. particle size and shape, suspension density, solute concentration and temperature, have an effect on the Raman spectra. Since these factors are changing during a (pseudo-)polymorphic transformation process, a detailed understanding of their influences on the quantitative application of Raman spectroscopy is required.

Generally, liquid phase composition can be measured in situ by application of several spectroscopic techniques, e.g. near-\(^{61,62}\) and mid-infrared\(^{14–17,58,67}\) and Raman spectroscopy\(^{22,64}\). However, obtaining accurate and robust solute concentration estimations has shown to be challenging for nonisothermal processes involving substances with low solubilities such as L-glutamic acid in an aqueous environment. Real-time characterization of the liquid and solid phase properties enables estimation of the kinetics of the different phenomena that govern a solvent-mediated polymorph transformation process, i.e. the nucleation, growth and dissolution rates of the involved solid forms\(^{68,69}\).

In this study, ATR-FTIR and Raman spectroscopy have been employed to measure liquid and solid phase composition in situ. Both spectroscopic techniques were applied quantitatively by means of multivariate
data analysis, so-called chemometric methods, to overcome complex and
time-consuming procedures such as the selection of representative sig-
nals and peak deconvolution. For both ATR-FTIR and Raman spec-
troscopy, accurate and robust concentration estimations were obtained
and a broad applicability of this approach was demonstrated through
experiments covering a wide range of process conditions. Additionally,
it was demonstrated that multivariate analysis of Raman data allows for
solute concentration estimation despite the fact that solute signals are
weak and completely overlapping with signals related to the solid phase.

4.2 Experimental

4.2.1 Materials and methods

L-glutamic acid monosodium salt monohydrate (≥ 98 %, Sigma-Aldrich,
Buchs, Switzerland), fuming hydrochloric acid solution (37 – 38 %,
Sigma-Aldrich, Buchs, Switzerland) and deionized water were used for all
experiments. L-glutamic acid has two known polymorphs, a meta-
stable α and a thermodynamically stable β polymorph. Whereas the
β-polymorph was purchased (≥ 99 %, Sigma-Aldrich, Buchs, Switzer-
land), the α polymorph is not commercially available and was obtained
via pH-shift precipitation by mixing a 0.4 molar monosodium mono-
hydrate solution with a 0.4 molar hydrochloric acid solution at 5°C.
The crystals were filtered 45 minutes after the onset of particle forma-
tion. The polymorphic form was verified using powder X-ray diffraction
as shown in Figure 4.1. The meta-stable prismatic α polymorph can
also be clearly distinguished visually from the thermodynamically stable
needle-shaped β polymorph as can be seen in the insets of Figure 4.1.
Figure 4.1: Powder X-ray diffraction patterns of metastable $\alpha$ and stable $\beta$ polymorph, the insets show microscopy images of both polymorphs.

The details about the in situ and the ex situ characterization techniques that were applied to obtain the reported results can be found in Section 2.2 and Section 2.3, respectively.

### 4.2.2 Experimental design

**ATR-FTIR spectroscopy**

The ATR probe is designed to probe exclusively liquid phase in the presence of solid material, hence only liquid properties such as composition, temperature and viscosity are expected to influence the infrared absorbance. However, temperature variations influence the baseline of the measured infrared signal, hence a baseline correction is required to compensate for the temperature effect. A univariate peak height or area calibration is in principle able to quantify liquid composition even for nonisothermal processes. However, variable selection and a proper baseline correction can be complex and multivariate models are expected to
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

result in more accurate and robust solute concentration estimations\(^\text{15}\). Figure 4.2 shows the influence of concentration (a) and temperature (b) on the infrared signal for a L-glutamic acid solution in water. As expected, the absorbance increases for an increasing concentration and a linear baseline correction is able to compensate for the temperature effect.

![Figure 4.2: Influence of the solute concentration at 35°C (a) and of the temperature at 15.05 g/kg (b) on the infrared absorbance](image.png)

In order to obtain a calibration model that is applicable over a wide range of process conditions, an extensive calibration set was designed that covered samples in the under- as well as in the supersaturated region. An undersaturated solution was prepared by dissolving a known amount of the $\beta$-form. Spectral acquisition was started after the FBRM counts reached a minimum and the infrared signal remained constant, thus indicating complete dissolution. A cooling rate of 5°C/hr was applied, during which spectra were recorded every two minutes. A rapid increase in counts of the FBRM signal indicated the onset of particle formation as the operating point crosses the metastable zone limit; subsequent samples were rejected from the calibration set. This pro-
4.2 Experimental

Procedure was repeated for 5 concentrations covering the range of process conditions of interest, thus resulting in a calibration set consisting of 500 calibration data points. Figure 4.3 shows the temperature and the concentration of the samples included in the calibration set together with the solubility curve of $\beta$-L-glutamic acid$^{58}$. Variable selection was based on the characteristic absorption bands of L-glutamic acid, i.e. $1280 - 1480 \text{ cm}^{-1}$, thus resulting in 105 variables. In order to improve the accuracy and robustness of the calibration model, a scaled temperature (maximum absorbance multiplied by temperature and divided by the maximum temperature, both in degrees Celsius) of each calibration sample was included as an additional variable. Due to signal drifts induced by external effects that can occur during long monitoring periods, a linear baseline correction, i.e. a first order polynomial, was required to obtain consistent results.

![Figure 4.3: Temperature and concentration of calibration samples (○) used for ATR-FTIR monitoring and solubility curve (■) of L-glutamic acid$^{58}$. To enhance the visibility only half of the data points is displayed.](image)
Raman spectroscopy

Raman scattering results from both the solid and the liquid phase, hence properties of both phases have to be considered for the quantitative application of Raman spectroscopy in heterogeneous processes such as crystallization, thus making it particularly challenging\textsuperscript{55}. In a solid-state transformation process, the solid composition is the major process variable influencing qualitative and quantitative features of the Raman spectra. However, also suspension density, solute concentration, particle size and temperature are expected to influence quantitative features of the Raman spectra. Since the suspension density and the particle size distribution change significantly during a solid-state transformation process, this additional variability should be investigated thoroughly.

Figure 4.4 illustrates the effect of solid composition (a) and solute concentration (b) on the Raman spectrum. Different characteristic peaks for the two polymorphs can be distinguished and employed to quantify the solid composition\textsuperscript{58,63}. As it can be seen in Figure 4.4(b), the characteristic bands of L-glutamic acid in solution increase for an increasing liquid phase concentration and could in principle be used for solute concentration estimation\textsuperscript{22,64}. However, in a heterogeneous process, the solute signals overlap completely with the solid signals and cannot be employed for quantitative application through peak area or peak height calibration methods.

In order to gain insight into the effect of particle size on Raman spectroscopy and to perform seeded polymorph transformation experiments, different particle size distributions were obtained by wet sieving $\alpha$ crystals. The obtained fractions and corresponding particle size distributions are shown in Figure 4.5. Subsequently, all sieve fractions were analyzed as dry powder as well as in a saturated suspension at 25°C. Figure 4.6
4.2 Experimental

Figure 4.4: Influence on Raman spectroscopy of solid composition (a) at 2 wt.% of total suspension density and 25°C, the solute concentration was equal to the solubility of the α form except for the 100 wt.% β polymorph suspensions. Influence of the solute concentration on Raman spectroscopy at 25°C (b) for concentrations of 0, 21.7, 38.0, 43.4, 54.3, 65.1 g/kg. The arrow indicates an increasing solute concentration.

shows that the Raman signal intensity of different characteristic peaks is decreasing for increasing particle size. This observation is in agreement with previous studies\textsuperscript{65,70,71}, however it disagrees with the general theory of diffuse reflection spectroscopy as developed by Kubelka and Munk (1931)\textsuperscript{19}, which predicts a decreasing intensity for a decreasing particle size. As stated in the literature\textsuperscript{71}, the established theoretical framework assumes confocal optics, whereas the immersion probes applied in this work are characterized by non-confocal optics. A decreasing particle size will increase the light scattering leading to an increased Raman scattering volume which results in an increase in the Raman signal intensity\textsuperscript{71}. Because light scattering depends on the shape of the scattering particles as well, the crystal shape is also expected to influence the Raman signal intensity. This relationship between the particle size and the Raman signal intensity has been employed to characterize the mean diameter in
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

Figure 4.5: Particle size distribution of α crystals obtained by wet sieving. The displayed numbers indicate the size ranges of the sieves that were used.

The displayed numbers indicate the size ranges of the sieves that were used.

Case of the crystal size\textsuperscript{65} and polymer particle size\textsuperscript{72}.

The effect of suspension density for β-L-glutamic acid crystals at 25°C is illustrated in Figure 4.7. As it can be seen, characteristic signals increase significantly for an increasing suspension density.

Temperature effects were expected to be negligible at the mild process conditions\textsuperscript{19} applied in this work and were indeed found to be minimal.

In order to include variation due to the aforementioned process variables, an extensive calibration set was designed based on measurements in suspension. Calibration samples were prepared by adding different amounts of a solid powder mixture to a saturated solution at a certain temperature. Since an extensive experimental design covering all process variables would require thousands of samples, a significantly shorter approach was applied here. The solid composition being the most important process variable, it was included at 11 levels, i.e. 0, 5, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 95 and 100 wt.% α polymorph using the same suspension density. Each calibration sample was measured three times and this procedure was performed at 25°C and at 45°C. Additionally,
4.2 Experimental

Figure 4.6: Influence of particle size on Raman signal intensity in solid state (a) and suspension (b) indicated by the baseline corrected height of two characteristic peaks. All measurements were performed using α polymorph particles at 25°C and for the suspension measurements the suspension density was 2 wt.% and solute concentration was equal to the solubility of the α form.

Figure 4.7: Influence of the suspension density on the Raman signal intensity. The arrow indicates an increasing suspension density of β L-glutamic acid crystals at 25°C, (0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 wt.%).
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

different suspension densities were prepared at 25°C and at 45°C using exclusively α or β polymorph. Different particle sizes for α and β crystals were used to prepare the calibration samples. Table 4.1 summarizes the calibration set used for quantitative application of Raman spectroscopy.

4.2.3 Multivariate data analysis

Multivariate data analysis techniques such as principal component analysis and partial least squares regression were employed in order to facilitate data interpretation and enable quantitative application of in situ ATR-FTIR and in situ Raman spectroscopy. For a detailed discussion, the reader is referred to Section 3.1.

4.3 Results and discussion

4.3.1 ATR-FTIR spectroscopy

Calibration

Table 4.2 summarizes the data analysis of the calibration set for ATR-FTIR spectroscopy. The presence of collinearity, i.e. the fact that numerous variables are correlated and provide essentially the same information, can be verified through several numerical and statistical criteria. The determinant and condition number, i.e. the ratio between the largest and smallest singular value of a matrix, in this case $X^TX$, are the most frequently used among these criteria. It is worth noting that the matrix $X^TX$ is used in standardized form, i.e. the matrix $X$ has been scaled so
Table 4.1: Calibration set as used for the quantitative application of Raman spectroscopy. \( w_\alpha \): fraction of \( \alpha \) polymorph in total amount of solid; \( w_s \): suspension density; \( c_s \): solute concentration; \( T \): temperature; \( d_{43}^\alpha \), \( d_{43}^\beta \): particle size of \( \alpha \), \( \beta \) polymorph respectively. \( c_\alpha^* \) and \( c_\beta^* \) indicate the solubility of the \( \alpha \) and \( \beta \) polymorph respectively. \( w_{\alpha, var} \) indicates the different solid compositions: 0, 5, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 95, 100 wt.% of \( \alpha \) polymorph.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( w_\alpha ) [wt.%]</th>
<th>( w_s ) [wt.%]</th>
<th>( c_s ) [g/kg]</th>
<th>( T ) [°C]</th>
<th>( d_{43}^\alpha ) [µm]</th>
<th>( d_{43}^\beta ) [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–33</td>
<td>( w_{\alpha, var} )</td>
<td>2</td>
<td>( c_\alpha^* )</td>
<td>25</td>
<td>112.5</td>
<td>42.0</td>
</tr>
<tr>
<td>33–66</td>
<td>( w_{\alpha, var} )</td>
<td>2</td>
<td>( c_\alpha^* )</td>
<td>45</td>
<td>197.8</td>
<td>79.3</td>
</tr>
<tr>
<td>67–84</td>
<td></td>
<td>2, 2.5, 3.5, 4, 5, 6.5</td>
<td>( c_\beta^* )</td>
<td>25</td>
<td>-</td>
<td>79.3</td>
</tr>
<tr>
<td>85–102</td>
<td>0</td>
<td>2, 2.5, 3.5, 4, 5, 6.5</td>
<td>( c_\beta^* )</td>
<td>45</td>
<td>-</td>
<td>79.3</td>
</tr>
<tr>
<td>103–117</td>
<td>100</td>
<td>4.5, 5, 6.5</td>
<td>( c_\alpha^* )</td>
<td>25</td>
<td>298.4</td>
<td>-</td>
</tr>
<tr>
<td>118–142</td>
<td>100</td>
<td>1.5, 2, 3, 4.5, 5</td>
<td>( c_\alpha^* )</td>
<td>45</td>
<td>197.8</td>
<td>-</td>
</tr>
<tr>
<td>143–167</td>
<td>100</td>
<td>1.5, 2, 3, 4.5, 5</td>
<td>( c_\alpha^* )</td>
<td>45</td>
<td>352.6</td>
<td>-</td>
</tr>
</tbody>
</table>
that the sum of the squares of each column vector equals one, which is necessary if the variables are of very different magnitudes. In case the determinant is smaller than $10^{-3}$ and the condition number exceeds $10^3$ there is good evidence for collinearity$^{73}$. For the calibration set as used for infrared spectroscopy, the determinant and condition number of $X^TX$ were in fact 0 and $6.4 \times 10^{17}$, respectively, thus making decomposition techniques such as PCR and PLSR necessary. Two different characteristic peaks, at $1354 \text{ cm}^{-1}$ and $1408 \text{ cm}^{-1}$, have been selected and their peak height and peak area were related to solute concentration. Furthermore, multivariate models were applied on a broad spectral range as described in Section 4.2.2. As readily observed in Table 4.2, peak height and peak area calibration models are less accurate than multivariate calibration models as indicated by the larger RMSECV and the smaller $R^2$ and $Q^2$ values. A better peak area calibration was obtained by using two peak areas as variables in a MLR calibration model. As expected, PLSR performs better than PCR applied on the same wavenumber range and using the same number of latent variables. In order to study the effect of temperature as an additional variable, a scaled temperature has been added as an additional column to matrix $X$ as explained in Section 4.2.2. However, the addition of temperature does not improve model performance significantly. The best model performance was found for a PLSR model using 6 latent variables, hence this was used for in situ monitoring purposes. The corresponding RMSECV was 0.186 g/kg which corresponds to 1.2 % of the mean of the concentrations as used in the calibration set.

**Solubility curve measurement**

To validate the calibration model, the solubility of the $\beta$ polymorph of L-glutamic acid in water was measured using in situ ATR-FTIR spec-
### Table 4.2: Data analysis results for infrared spectroscopy.

All data are mean-centered, each spectrum was smoothed using a moving average filter over 3 points and baseline corrected. The mean of the concentration used equalled 15.26 g/kg. LV: latent variable; RMSECV: root-mean-square error of cross-validation; \( R^2 \): correlation coefficient; \( Q^2 \): cross-validation coefficient. The * indicates that a scaled temperature has been incorporated as an additional variable.

<table>
<thead>
<tr>
<th>Method</th>
<th>Variables [cm(^{-1})]</th>
<th># LV’s</th>
<th>RMSECV [g/kg]</th>
<th>( R^2 )</th>
<th>( Q^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>peak height</td>
<td>( \lambda = 1354 )</td>
<td>-</td>
<td>0.841</td>
<td>0.979</td>
<td>0.979</td>
</tr>
<tr>
<td>peak height</td>
<td>( \lambda = 1408 )</td>
<td>-</td>
<td>0.739</td>
<td>0.984</td>
<td>0.984</td>
</tr>
<tr>
<td>peak area</td>
<td>( 1338 \leq \lambda \leq 1380 )</td>
<td>-</td>
<td>0.949</td>
<td>0.974</td>
<td>0.974</td>
</tr>
<tr>
<td>peak area</td>
<td>( 1380 \leq \lambda \leq 1440 )</td>
<td>-</td>
<td>0.736</td>
<td>0.984</td>
<td>0.984</td>
</tr>
<tr>
<td>peak area</td>
<td>( 1338 \leq \lambda \leq 1380 + 1380 \leq \lambda \leq 1440 )</td>
<td>-</td>
<td>0.648</td>
<td>0.988</td>
<td>0.988</td>
</tr>
<tr>
<td>PCR</td>
<td>( 1281 \leq \lambda \leq 1481 )</td>
<td>6</td>
<td>0.209</td>
<td>0.988</td>
<td>0.988</td>
</tr>
<tr>
<td>PLSR</td>
<td>( 1281 \leq \lambda \leq 1481 )</td>
<td>6</td>
<td>0.186</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>PCR*</td>
<td>( 1281 \leq \lambda \leq 1481 )</td>
<td>6</td>
<td>0.206</td>
<td>0.988</td>
<td>0.988</td>
</tr>
<tr>
<td>PLSR*</td>
<td>( 1281 \leq \lambda \leq 1481 )</td>
<td>6</td>
<td>0.187</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

troscopy and compared to known solubility data. A saturated suspension was heated from 20°C to 60°C at a rate of 1°C/hr. At 30°C, 40°C and 50°C the temperature was held constant for 3 hours to verify equilibrium conditions during the entire course of the experiment. Crystals were added to the suspension in order to minimize the probability of solid particles sticking to the ATR probe window, thereby hindering liquid phase measurements. Figure 4.8 shows the temperature profile and the estimated liquid phase concentration (a) as well as the measured solubility curve (b), which exhibits excellent agreement with literature data even at very low concentrations. It must be noted that the influence of NaCl on the solubility was found to be negligible for L-glutamic acid. This approach enables nonisothermal measurements of the solubility, whereas in previous studies suspensions were equilibrated at a certain temperature and concentrations were measured at a limited number of temperatures. This approach is general and was applied successfully to different substances, as is illustrated in Figure 4.9 where the solubility curves of oxalic acid in water (a) and of paracetamol in water (b) are shown and compared with gravimetrically determined solubility values.

Cooling crystallization

To enable on-line control of cooling crystallization processes, accurate in situ measurement of the solute concentration is a primary requirement. The approach presented in this work can be used for this purpose and this is demonstrated by a series of desupersaturation experiments using the β form of L-glutamic acid. Saturated suspensions were obtained by equilibration for several hours at 50°C, and subsequently cooled at different linear cooling rates down to 25°C followed by a temperature plateau. To ensure that no significant nucleation occurred and thereby that the
4.3 Results and discussion

Figure 4.8: Measured solubility of $\beta$ L-glutamic acid in water (○) as function of time (a) and temperature (b). In (b) the (○) represent solubility measurement by ATR-FTIR\textsuperscript{58} and gravimetrically determined solubilities are shown as (□)\textsuperscript{74}.

Figure 4.9: Measured solubility curve for oxalic acid in water (a) and paracetamol in water (b). In both figures measured solubility using ATR-FTIR spectroscopy is compared with gravimetrically determined solubility data shown as (□).
supersaturation was consumed exclusively by crystal growth, the FBRM was employed to verify that there was no increase in counts in small size ranges. For higher cooling rates than those discussed here, nucleation was detected by FBRM and solid particles sticking to the ATR probe window corrupted the ATR-FTIR measurements. Figure 4.10(a) shows the concentration profiles as a function of temperature; it can be seen that for higher cooling rates higher concentrations and thereby supersaturations were achieved. Figure 4.10(b) shows the supersaturation profiles as a function of time. Clearly, the achieved supersaturation increases for an increasing cooling rate and supersaturation is gradually consumed when the final temperature is reached and maintained. The fact that supersaturation does not reach unity is in agreement with earlier studies and can be due to the presence of impurities. The scattering of the supersaturation profiles after the maximum supersaturation is reached and the temperature is kept constant is due to the fact that concentrations are very low, i.e. 1 wt.% or lower.

Figure 4.10: Concentration (a) and desupersaturation profiles (b) with respect to the β polymorph of L-glutamic acid in water for different cooling rates. The suspension density at 50°C equalled 0.5 wt.% in all experiments.
4.3 Results and discussion

**Polymorph transformation**

It is known from previous studies\textsuperscript{52,58,75} that crystals can stick to the ATR probe window, ultimately leading to complete clogging. In this work, solid interferences were encountered during unseeded as well as seeded polymorph transformation experiments. Cleaning the probe window as soon as the presence of solid is detected in the infrared spectrum might be a solution for relatively short experiments, however, for longer experiments, this is not a feasible approach. Besides, multivariate calibration is expected to be more sensitive to solid interferences than peak area or peak height calibration. Figure 4.11(a) shows the solute concentration for an unseeded polymorph transformation at 45\textdegree{}C with an initial supersaturation of 3.0 with respect to the $\alpha$ polymorph. It can be readily observed that the initial concentration is estimated correctly, however, after the formation of $\alpha$ crystals (as verified through Raman spectroscopy) the measured infrared spectra exhibit interferences of solid material as shown in Figure 4.11(b). Clearly, four distinct signals due to solid material can be distinguished. After the last $\alpha$ crystal detaches from the probe window, the concentration estimation equals the solubility of the $\beta$ polymorph as is expected after the completion of the solid-state transformation\textsuperscript{58}.

4.3.2 Raman spectroscopy

**Exploratory data analysis**

To provide a qualitative idea of the solid-state transformation and the resulting Raman data, the time-resolved Raman spectra in the characteristic range are displayed for a seeded and unseeded transformation experiment in Figures 4.12(a) and 4.12(b), respectively. As it can be
4. Quantitative application of in situ ATR-FTIR and Raman spectroscopy

Figure 4.11: Solute concentration during an unseeded polymorph transformation $S_\alpha = 3.0$ at 45°C (a) and evidence of solid interferences due to $\alpha$ crystals sticking on the ATR window (b). The arrows indicate the characteristic signals for solid material.

seen, the peak characterizing the solid phase shifts gradually from 870 to 865 cm$^{-1}$ as the $\alpha$ form transforms into the $\beta$ form. Concerning Figure 4.12(b), the formation of crystals can be readily observed by the drastic change in the Raman spectra during the first hour of the experiment, i.e. a strong increase in Raman intensity at 870 cm$^{-1}$ due to the solid phase. Moreover, the depletion of supersaturation can be observed by a strong decrease in Raman intensity in the characteristic range for the solute, i.e. at 857 cm$^{-1}$. It should be emphasized that after the nucleation of the $\alpha$ form and the depletion of supersaturation, the unseeded polymorphic transformation is governed by the same phenomena as the seeded solid-state transformation, i.e. dissolution of the $\alpha$ form and nucleation and growth of the $\beta$ polymorph, and is expected to yield similar qualitative and quantitative results. Since the solubility of the $\beta$ polymorph is lower than the solubility of the $\alpha$ form, the suspension density increases while the transformation proceeds. However, the Raman
signal intensity is decreasing during the transformation, indicating that more process variables than the suspension density influence the Raman signal intensity. This indicates the necessity of preprocessing techniques that can compensate for additional variability in the Raman spectra.

Exploratory data analysis on in situ measurements can provide qualitative information without extensive calibration procedures and without off-line characterization techniques such as powder X-ray diffraction. Here, seeded and unseeded polymorphic transformations were analyzed using principal component analysis (PCA) on a variable range characterized by $820 \leq \lambda \leq 1480$ cm$^{-1}$. Figures 4.12(c) and 4.12(d) show the scores, i.e. the individual columns of matrix $\mathbf{T}$ as defined in eq. (3.11), of the first three principal components (PCs) over time for the seeded and the unseeded polymorphic transformation, respectively. Obviously, the first principal component is related to the solid composition and a clear endpoint of the solid-state transformation can be determined in both cases. The physical interpretation of the second and third principal components is less obvious; they are most likely combinations of the effects of an increasing suspension density, a decreasing solute concentration and a changing particle size distribution. However, in the case of the unseeded solid-state transformation, more variation can be observed in the second principal component, representing the dramatic change in suspension density during particle formation. The third principal component in Figure 4.12(d) exhibits a similar behavior as the second PC in Figure 4.12(c). Figures 4.12(e) and 4.12(f) display the scores for the first three principal components in a three dimensional representation for the seeded and the unseeded transformation, respectively. It is worth noting that the axes corresponding to the second and the third principal component in the two figures have been exchanged.
As it can be readily observed in Figure 4.12(e), variation in the third principal component is negligible as compared to the first two principal components and the polymorphic transformation can be represented as a path in the PC1-PC2 plane as indicated by the curved arrows. As shown in Figure 4.12(f), the unseeded polymorphic transformation can be visualized also as a path in the three dimensional space, as indicated by the curved arrows. The different phases of the process, i.e. formation of nuclei at high supersaturation, transformation from \( \alpha \) to \( \beta \) form, and equilibrium at the solubility of the \( \beta \) polymorph, can be distinguished. After the formation of particles and the depletion of supersaturation, a similar trajectory can be observed for the unseeded transformation in the PC1-PC3 plane as for the seeded transformation in the PC1-PC2 plane. In both figures, the endpoint of the transformation can be identified by a high density of measurement points. This method provides qualitative information about a solid-state transformation process without extensive calibration procedures or off-line characterization. A similar approach was applied by others using in situ near infrared spectroscopy\(^{76}\).

**Calibration**

In order to apply Raman spectroscopy quantitatively, a multivariate data analysis has been performed using an extensive calibration set as described in Section 4.2.2. Different variable ranges were tested based on the characteristic signals of L-glutamic acid. Raman shifts ranging from 820 to 1480 cm\(^{-1}\) were found to result in the lowest prediction errors, hence this range was applied in all further analyses. Table 4.3 summarizes the multivariate analysis for the estimation of solid composition as well as of solute concentration.
4.3 Results and discussion

Figure 4.12: Time-resolved Raman spectra for a seeded (a) polymorphic transformation using 30 g/kg seeds with a characteristic size $d_{43}$ of 197.8 µm at 45°C and an unseeded (b) polymorphic transformation at 45°C with an initial supersaturation $S_\alpha = 2.5$. Scores over time and in a three dimensional representation for the seeded (c,e) and unseeded (d,f) polymorphic transformations. The numbers in (c) and (d) represent the variance captured in the particular principal component. The curved arrows in (e) and (f) indicate the direction of the transformation.
Concerning the solid composition, normalization of the spectra, i.e. the sum of all intensities equals unity, and application of a baseline correction significantly increase model performance as indicated by the lower RMSECV and the higher $Q^2$ and $R^2$ values. Standard normal variate transformation and multiplicative scatter correction result in a further increase in model performance. Similar trends can be observed for the PLSR and PCR calibration models. As in the case of multivariate analysis of ATR-FTIR data, PLSR performs better than PCR models. As reported in Table 4.3, a PLSR model using standard normal variate transformed data resulted in the lowest prediction errors, i.e. 3.229 wt.% $\alpha$ polymorph, hence this model was used for further monitoring purposes.

For solute concentration estimation, it was found that all preprocessing techniques decrease model performance and untreated Raman spectra resulted in the best model performance, i.e. 0.943 g/kg. Again, PLSR models perform better than PCR calibration models. As expected, prediction errors are larger than in the case of ATR-FTIR spectroscopy. Nevertheless, Raman spectroscopy enables simultaneous measurement of both the solid and the liquid composition, i.e. with only one instrument one can obtain information on both phases.

**Quantitative application**

In order to validate the Raman calibration model, it was applied to the seeded and unseeded polymorphic transformations as discussed above. Figure 4.13(a) shows the estimations of liquid and solid phase composition for the seeded transformation experiment as shown in Figure 4.12(a). The initial solid composition starts at 100 wt.% $\alpha$ polymorph and the solid gradually transforms into 100 wt.% $\beta$ polymorph.
### Solid

<table>
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### Solute

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Table 4.3: Data analysis results of Raman spectroscopy. All data are mean-centered and data used for solute concentration quantification was corrected for an off-set. LV: latent variable; norm.: normalization; bsl: baseline; RMSECV: root-mean-square error of cross-validation; $R^2$: correlation coefficient; $Q^2$: cross-validation coefficient.
Solute concentration monitoring displays a rapid decrease in concentration at the end of the solid-state transformation, thus indicating fast dissolution of the α form and relatively slow nucleation and growth of the β form\textsuperscript{58}. Figure 4.13(b) plots the liquid and the solid composition for the unseeded transformation experiment as discussed above and shown in Figure 4.12(b). During the first 30 minutes or so the solute concentration remains constant; this period is the induction period during which nuclei form and grow to a detectible size. The solute concentration prior to particle formation is underestimated, which is not unexpected since calibration samples covered exclusively suspension measurements having significantly different spectra as compared to solution measurements. The initial supersaturation was created by mixing a monosodium monohydrate solution with a hydrochloric acid solution of known composition, thereby initial supersaturation is known. The validity of this method to create supersaturation was verified in Section 4.3.1 where initial supersaturation was estimated correctly using ATR-FTIR spectroscopy and a multivariate calibration model. The onset of particle formation can be identified by a rapid decrease in solute concentration and the formed crystals are characterized as 100 wt.% α polymorph. Then, the solid composition remains nearly constant for approximately five hours, after which it changes due to dissolution of the unstable α polymorph and nucleation and growth of the stable β polymorph. The solute concentration decreases during this period from the solubility of the α form to the solubility of the β form. After approximately 12.5 hours the solid-state transformation is completed and 100 wt.% of the β polymorph is present. For both polymorphic transformations, the absolute solid concentration can easily be obtained through an overall mass balance. These experiments are the starting point of a study on seeded and unseeded solvent-mediated polymorphic transformation in
4.4 Conclusions

Figure 4.13: Seeded polymorph transformation experiment (a) using 30 g/kg seeds with a characteristic size $d_{43}$ of 197.8 µm at 45°C. Unseeded polymorph transformation experiment (b) at 45°C with an initial supersaturation with respect to the α polymorph equal to 2.5.

which characteristic seed size and mass, temperature and initial supersaturation are varied. In that work the liquid and the solid phase data obtained using the multivariate approach as described in this work were used to estimate the transformation kinetics, i.e. the nucleation, growth and dissolution rates of the involved solid forms, by means of a population balance model thereby enabling process design, optimization and control.

4.4 Conclusions

This chapter demonstrates the quantitative application of in situ ATR-FTIR and Raman spectroscopy using multivariate data analysis techniques in the case of crystallization and precipitation, i.e. multiphase processes under non-steady state conditions.

In situ ATR-FTIR spectroscopy has been applied to monitor liquid com-
position. It was shown that a significantly better quantitative model was obtained by multivariate analysis of ATR-FTIR data as compared to standard univariate approaches based on peak height or area. Through variable selection and data preprocessing techniques, an accurate and robust solute concentration estimation was obtained even for a sparingly soluble substance such as L-glutamic acid in aqueous solution. The general and robust applicability of this method was demonstrated via solubility curve measurements for different substances, i.e. β-L-glutamic acid, oxalic acid and paracetamol, and nonisothermal desupersaturation measurements for β-L-glutamic acid. In this study, crystals of the α polymorph of L-glutamic acid were observed to attach to the ATR probe window, thereby hampering correct solute concentration measurements. However, we believe that ATR-FTIR spectroscopy in combination with multivariate data analysis is of great importance for an accurate in situ measurement of liquid phase composition, especially when measuring in a suspension as in crystallization processes.

The Raman scattering signal emerges from both the liquid and the solid phase, hence there are numerous factors influencing it, which make the quantitative application of this technique more challenging. In this work, a significant increase in Raman signal intensity for a decreasing particle size was observed which is consistent with previous studies. On the one hand the effect of solid and liquid phase properties enables in principle the measurement of solid and liquid composition simultaneously, but on the other hand it represents a drawback of Raman spectroscopy that requires more advanced calibration and preprocessing methods in order to be applied in a quantitative manner. It was shown that principal component analysis of Raman data enables fast end-point determination of a solid-state transformation with little calibration efforts. An experimental design, covering process conditions that influence the
4.4 Conclusions

Raman signal, has been proposed that allows for a quantitative application of Raman spectroscopy. It was demonstrated that multivariate data analysis requires preprocessing techniques, such as standard normal variate transformation, improving model performance significantly. The applicability of this approach for the quantification of solid-state composition was shown for seeded and unseeded polymorph transformation experiments under different process conditions in terms of temperature, suspension density and particle size of seed crystals. Additionally, it was shown that multivariate data analysis enables also the monitoring of the solute concentration despite the fact that solute signals in the Raman spectra are weak and completely overlapping with signals associated to the solid phase. This demonstrates that one in situ technique, i.e. Raman spectroscopy, enables simultaneous measurement of the solid as well as of the liquid composition.
Chapter 5

Monitoring and modeling the polymorph transformation of L-glutamic acid

In the first part of this chapter, the polymorph transformation of the metastable α to the stable β polymorph of L-glutamic acid at 45°C was monitored using in situ Raman spectroscopy combined with a calibration. In a series of seeded transformation experiments the effect of


Cornel, J.; Mazzotti, M. Calibration-free quantitative application of in situ Raman spectroscopy to a crystallization process. *Analytical Chemistry* 2008, 80 (23), 9240-9249.
different operating conditions on the transformation was studied. Both
increasing seed mass and increasing stirring rate decrease the transfor-
mation time, thus suggesting an attrition-based secondary nucleation
mechanism of the polymorph. Moreover, it was found that no pure seed
crystals of the metastable $\alpha$ polymorph could be produced and that dif-
ferent sieve fractions of the $\alpha$ polymorph contained different amounts of
the $\beta$ polymorph, which was included within the $\alpha$ crystals. These inclu-
sions had a significant effect on the transformation times meaning that
in experiments with larger seeds the transformation was faster than in
experiments with smaller seeds. Independent seeded batch desupersatu-
ration experiments were conducted to determine the growth rate of the
$\beta$ polymorph. On the basis of this growth rate and of the seeded trans-
fornations, the secondary nucleation rate of the $\beta$ form was estimated
using a process model. Together with nucleation and growth kinetics of
the $\alpha$ polymorph, which were measured previously, a fully descriptive
model of the polymorph transformation process was developed.

In the second part of this chapter, the data obtained using in situ Ra-
man spectroscopy to monitor this polymorph transformation has been
used in a quantitative manner without the use of a calibration. Ass-
suming a linear dependency of the Raman signal intensity on the solute
and on the solid concentrations of both solid-state forms, the measured
time-resolved Raman spectra are fitted directly using a detailed model
that describes the time-evolution of the process. The applicability of
this novel method is demonstrated thoroughly through the application
to synthetic data of unseeded and seeded transformations as well as to
various seeded polymorph transformation experiments. The resulting
concentration profiles show a good agreement with the concentrations
obtained by a multivariate calibration model. Additionally, the esti-
mated kinetic parameters are compared to parameters obtained through
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

fitting the solid phase composition profiles that result from the calibration. The discrepancy between the estimated model parameters is small and essentially the same descriptive process model is obtained. However, by fitting the time-resolved Raman spectra directly, a significant amount of calibration effort can be avoided.

5.1 Calibration-based approach

5.1.1 Introduction

Polymorphism is of key importance in the pharmaceutical and fine-chemical industry since different polymorphs have different physical and chemical properties, such as solubility and reactivity. A process involving multiple polymorphs simultaneously, i.e. concomitant polymorphism, can produce more than one crystalline form or a mixture of different crystal structures depending on the operating conditions. To avoid production of undesired polymorphs and to obtain a robust crystallization process, thermodynamics and kinetics must be known. Concerning the thermodynamics of a polymorph transformation, the Gibbs free energy of the involved polymorphic forms as a function of temperature determine if only one or multiple stable polymorphs exist. A compound is called monotropic or enantiotropic depending whether the same polymorph or different polymorphs, respectively, are stable at different temperatures. The kinetics of nucleation and growth of each polymorph determines which appears first and how long the transformation from the one, the metastable form, to the other, the stable form, takes. The overall kinetics results from a complex interplay between the involved physical phenomena, i.e. nucleation, growth and dissolution of the involved solid-state forms. Ultimately, accurate kinetic information allows
process modeling and enables process design, optimization and control. Whereas the thermodynamic aspects of a solid-state transformation are relatively straightforward and simple to determine, the characterization of transformation kinetics has shown to be a nontrivial task.\textsuperscript{58,66,69,79–81}

The development of in situ monitoring techniques via spectroscopic, laser (back) scattering techniques or imaging, enables in situ characterization of the liquid and the solid phase and results in a significant increase in the amount of information about a particular process. Although the number of publications that report utilization of in situ characterization tools has increased significantly over the past decade,\textsuperscript{6,7,57,82} the measured quantities are rarely used to develop descriptive process models. Numerous publications describe a polymorphic transformation process quantitatively, however few consider in detail the underlying phenomena, i.e. nucleation, growth and dissolution of the individual solid-state forms. By employing population balance equations (PBEs) a wide variety of transformation processes can be described. Combination of PBE modeling with optimization routines enables estimation of unknown parameters characterizing the involved phenomena. Although PBEs and their numerical solution methods are well-known, few authors consider PBEs as a framework to model a polymorphic transformation. In a previous work,\textsuperscript{58} a PBE model of the solvent-mediated polymorphic transformation of L-glutamic acid was presented. However, the effect of various process conditions was not investigated. In a comparable study,\textsuperscript{69} the same transformation was considered and a descriptive process model was obtained. Yet, a complete insight in the secondary nucleation mechanisms was not provided. Recently, a detailed and thorough work was published considering the solvent-mediated anhydrous to monohydrate transformation of citric acid.\textsuperscript{81,83} The nucleation and growth rates were determined independently based on an appropriate number of exper-
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

ments. Secondary nucleation mechanisms were suggested to govern transformation kinetics and a descriptive model was developed.

In this work, we applied Raman spectroscopy to characterize the liquid as well as the solid phase in situ by means of multivariate data analysis techniques. Nucleation and growth rates of the $\alpha$ form were determined independently leaving the growth and nucleation rates of the stable $\beta$ polymorph to be determined through seeded batch desupersaturation and polymorph transformation experiments at different process conditions, i.e. stirring rate, seed mass and seed size.

5.1.2 Materials and methods

Materials

L-glutamic acid monosodium salt monohydrate (98 %, Sigma-Aldrich, Buchs, Switzerland), fuming hydrochloric acid solution (37 - 38 %, Sigma-Aldrich, Buchs, Switzerland) and deionized water were used for all experiments. L-glutamic acid has two known polymorphs, a meta-stable $\alpha$ and a thermodynamically stable $\beta$ polymorph. Whereas the $\beta$-polymorph was purchased (99 %, Sigma-Aldrich, Buchs, Switzerland), the $\alpha$ polymorph is not commercially available and was obtained via pH-shift precipitation by mixing equal amounts of a 0.4 molar monosodium monohydrate solution with a 0.4 molar hydrochloric acid solution at $5^\circ$C. The crystals were filtered 45 minutes after the onset of particle formation. The polymorphic form was verified using powder X-ray diffraction. The meta-stable prismatic $\alpha$ can also be clearly distinguished from the stable needle-shaped $\beta$ polymorph by optical microscopy. L-glutamic acid is a monotropic compound, where the $\beta$ polymorph is the thermodynamically stable solid-state form.
5.1 Calibration-based approach

**Experimental setup and characterization techniques**

All experiments were performed in the 100 mL volume reactor as described in Section 2.1 to reduce the amount of α crystals required for the seeded transformation experiments. All in situ, i.e. Raman, ATR-FTIR spectroscopy and FBRM measurements, and ex situ, i.e. multisizer measurements and powder X-ray diffraction, characterization techniques are described in Chapter 2. The calibration for Raman and ATR-FTIR spectroscopy has been presented in Chapter 4. In this work, the FBRM has been applied only to verify that no significant nucleation occurred during desupersaturation experiments. This would in fact be evident as it would yield a sudden increase in the small chord lengths, i.e. 1-10 µm, which has never been observed in the experiments reported in this work.

**Experimental procedures**

Seeded batch desupersaturation experiments were used to estimate growth kinetics of the β polymorph at 45°C. Two different sieve fractions of β L-glutamic acid were produced to estimate the growth kinetics. The initial PSDs of the seed crystals were measured using the Coulter Multisizer and are shown in Figure 5.1. In each experiment the seeds are added to a supersaturated solution and the change of supersaturation over time, also called desupersaturation profile, is monitored using ATR-FTIR spectroscopy as discussed elsewhere. The supersaturation was created by mixing equimolar solutions of monosodium glutamate and of hydrochloric acid. The experimental conditions are given in Table 5.1.
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

Figure 5.1: Particle size distributions of $\beta$ seeds used for growth rate experiments. The average particle sizes were $d_{43}(F_{\beta,1}) = 41 \, \mu m$ and $d_{43}(F_{\beta,2}) = 132 \, \mu m$.

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<th>seed fraction $\beta$</th>
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Table 5.1: Experimental conditions of the seeded batch desupersaturation experiments at 45°C. Each experiment was repeated once.
To elucidate the governing mechanisms and to estimate the parameters in the nucleation rate expression of the $\beta$ polymorph of L-glutamic acid, an extensive set of seeded transformation experiments was performed. Different particle size distributions of $\alpha$ seed crystals were obtained via wet sieving. The obtained fractions and corresponding particle size distributions are shown in Figure 5.2. In seeded experiments a certain seed mass with known particle size distribution was added to a saturated solution with respect to the $\alpha$ form that had been prepared by mixing a monosodium monohydrate solution and a hydrochloric acid solution. Raman spectra acquisition was started upon addition of the seed crystals. All seeded transformation experiments were performed at $45^\circ$C under conditions reported in Table 5.2.

Figure 5.2: Particle size distributions of $\alpha$ seeds obtained by wet sieving. The following nominal size ranges of the sieves were used: 125 - 250 $\mu$m ($F_{\alpha,1}$ sieve fraction, $d_{43} = 188 \mu$m), 250 - 355 $\mu$m ($F_{\alpha,2}$ sieve fraction, $d_{43} = 303 \mu$m), > 355 $\mu$m ($F_{\alpha,3}$ sieve fraction, $d_{43} = 412 \mu$m).

In contrast to earlier works$^{58,69}$, where crash-cooling was applied, the desired supersaturation in unseeded experiments was created through addition of a hydrochloric acid solution to a monosodium monohydrate solution. The pH-shift method has two important advantages over crash-
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

Table 5.2: Experimental conditions of the seeded transformation experiments at 45°C. Each experiment was repeated at least once.

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<td>$F_{\alpha,1}$</td>
<td>30</td>
<td>3</td>
<td>300</td>
</tr>
</tbody>
</table>
cooling: 1) supersaturation is created nearly instantaneously since mixing times are short, whereas during crash-cooling supersaturation is reached over a relatively long time period resulting in nucleation occurring within a range of temperatures instead of at one specific temperature level. 2) Crystals do not appear on the reactor walls as in the case of crash-cooling. It is worth noting that the reproducibility of unseeded experiments was found to be relatively poor, probably due to a varying level of microscopic dust particles in the reactor resulting in varying heterogeneous nucleation rates.

5.1.3 Mathematical description of the polymorph transformation

The mathematical description of the polymorphic transformation of L-glutamic acid is based on population balance equations (PBEs). Combined with an optimization routine, the model allows for the estimation of unknown parameters in the kinetics expressions. A detailed discussion about population balance equations is given in Section 3.2 and in the case of this polymorph transformation there are two PBEs as in eq. (3.27), i.e. one for each polymorph. The initial and boundary conditions that apply to the PBEs and material balance are as in eq. (3.29), eq. (3.30) and eq. (3.31). Mixing effects are assumed to be negligible as discussed elsewhere.\(^7^9,^8^6\). Agglomeration effects could be neglected since supersaturation is low.\(^8^6\). The concentration of the solute \(c(t)\) can be obtained through the material balance and is given by the following equation:

\[
\frac{dc}{dt} = -3k_{v,\alpha}\rho_{\alpha}G_{\alpha} \int_{0}^{\infty} L^2 n_{\alpha} dL - 3k_{v,\beta}\rho_{\beta}G_{\beta} \int_{0}^{\infty} L^2 n_{\beta} dL \quad (5.1)
\]
where $\rho_i$ and $k_{v,i}$ are the solid density and the volume shape factor of the $i^{th}$ form, respectively. The densities of both polymorphs were assumed to be the same and equal to 1540 kg/m$^3$. Volume shapes factors of $\pi/6$ and 0.01 were assumed for the $\alpha$ and the $\beta$ polymorph, respectively.$^{52,58}$ The solubilities of both polymorphs were determined previously as a function of temperature$^{58,84}$. The solubilities at 45°C are 21.7 g/kg of solvent and 17.0 g/kg of solvent for the $\alpha$ and $\beta$ polymorph, respectively.

The nucleation kinetics of the $\alpha$ polymorph is determined based on induction times measured using ATR-FTIR and FBRM as discussed elsewhere$^{87}$. The nucleation rate at 45°C used in this model is given in the first row of Table 5.3. It is worth noting that a modified expression of eq. (3.50), i.e. including $S_\alpha$ before the exponent, was found to better describe the primary nucleation kinetics of the $\alpha$ form.

At low supersaturation levels the $\beta$ polymorph nucleates only in the presence of the $\alpha$ form, as verified by long-term experiments where no nucleation occurred after several days in a clear solution that was supersaturated with respect to $\beta$ and slightly undersaturated with respect to the $\alpha$ form. At such low supersaturations, i.e. $S_\beta < 1.28$, primary nucleation of the $\beta$ polymorph is negligible.$^1$ Therefore, secondary nucleation mechanisms govern the formation of $\beta$ nuclei. These mechanisms could include surface and attrition based nucleation. A lumped expression as in eq. (3.51) and eq. (3.52) for the secondary nucleation rate was proposed in the literature$^{1,88}$. For the four blade impeller as used in this work, the power number can be estimated as $N_p = 0.6^1$.

The effect of supersaturation could not be studied since during the transformation period the concentration stays constant at the solubility of the $\alpha$ polymorph. Thus, a simplified expression for the nucleation rate of $\beta$ was applied:

$$J_\beta = k_jn_{\alpha}^{k_j2}e^{k_j3}$$  \hspace{1cm} (5.2)
5.1 Calibration-based approach

where $m_\alpha$ is the mass of the $\alpha$ polymorph, and $k_{J1}$, $k_{J2}$ and $k_{J3}$ are empirical parameters. The nucleation rate in eq. (5.2) is given in $m^{-3} s^{-1}$, $m_\alpha$ in $g \ kg^{-1}$, and $\bar{\epsilon}$ in $m^2 s^{-3}$. Recently, a similar rate expression was published to model the transformation of the anhydrous form of citric acid to the monohydrate form$^{81,83}$. Yet, the effect of stirring was not investigated by these authors.

The growth mechanism of the $\alpha$ polymorph was found to be integration controlled and of the birth-and-spread type$^{52,89}$. The growth kinetics at 45°$C$ was measured earlier$^{52}$ and is implemented as given in the second row of Table 5.3.

It has been shown that in solvent-mediated polymorph transformations either dissolution or growth can be rate controlling$^{90}$. If during the transformation the liquid phase concentration stays at the solubility of the metastable dissolving polymorph, as shown later for L-glutamic acid, then the transformation is growth controlled. Dissolution of the $\alpha$ polymorph was estimated based on the Sherwood correlation and was incorporated as as given in the third row of Table 5.3$^{58}$. As shown previously$^{58}$, dissolution is not the rate-determining step in this transformation; hence a change of one order of magnitude in the coefficient given in the expression for the dissolution rate $D_\alpha$ did not change the simulation results.

Nucleation and growth rates of the $\alpha$ form are only implemented in case of unseeded transformation experiments where $S \geq 1$. If the concentration is lower than the solubility of $\alpha$, the dissolution rate $D_\alpha$ is used instead of the growth rate $G_\alpha$ and the nucleation rate $J_\alpha$ is set to zero.

Growth of the $\beta$ polymorph was found to be of the birth-and-spread type as well$^{89}$. However, the parameters in the growth rate expression were unknown and had to be estimated from seeded batch experiments as will
be demonstrated later. The following expression, which is a simplified version of eq. (3.54), was employed to express the growth rate of the $\beta$ polymorph as a function of the supersaturation $S_\beta$:

$$G_\beta = k_{G1}(S_\beta - 1)^{5/6} \exp \left( - \frac{k_{G2}}{S_\beta - 1} \right)$$

(5.3)

<table>
<thead>
<tr>
<th>mechanism</th>
<th>expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ nucleation, $J_\alpha$</td>
<td>$J_\alpha = 2.4 \times 10^8 S_\alpha \exp \left( - \frac{4.4}{\ln S_\alpha} \right)$</td>
</tr>
<tr>
<td>$\alpha$ growth, $G_\alpha$</td>
<td>$G_\alpha = 9.6 \times 10^{-7} (S_\alpha - 1)^{2/3} (\ln S_\alpha)^{1/6} \exp \left( - \frac{0.54}{\ln S_\alpha} \right)$</td>
</tr>
<tr>
<td>$\alpha$ dissolution, $D_\alpha$</td>
<td>$D_\alpha = 2.2 \times 10^{-4} (S_\alpha - 1)$</td>
</tr>
</tbody>
</table>

Table 5.3: Known mechanisms and their kinetics expressions at 45°C used in the population balance model.

The model equations that are described above were solved using the moving pivot technique proposed by Kumar and Ramkrishna 44 and was discussed in Section 3.2.2. In the case of seeded transformation experiments, $J_\alpha$ and $G_\alpha$ are set to zero since $S_\alpha$ is smaller than or equal to unity. It should be noted that the reactor volume was constant since all concentrations are given per cubic meter of solvent.

As discussed above, the growth rate parameters of the $\beta$ polymorph were estimated on the basis of seeded batch desupersaturation experiments with $\beta$ seed crystals. The unknown parameters were estimated using a nonlinear optimization algorithm that minimizes the sum of squared residuals between the experimental and the simulated values of the supersaturation of $\beta$. The model parameters are estimated by minimizing the sum of squared residuals SSR, written as

$$SSR = \sum_{i=1}^{N_e} \sum_{j=1}^{N_{d,i}} (S_{i,j}^{exp} - S_{i,j}^{sim})^2$$

(5.4)
5.1 Calibration-based approach

where \( N_e \) is the number of experiments, \( N_{d,i} \) denotes the number of data points per experiment \( i \), and \( S^{exp} \) and \( S^{sim} \) the experimental and simulated supersaturation with respect to the \( \beta \) form, respectively. The experimental data of seeded desupersaturation experiments given in Table 5.1 has been utilized. The Newton-Gauss-Levenberg/Marquardt (NGL/M) algorithm was used through the MATLAB \textit{lsqnonlin} function to solve the optimization problem.

The unknown parameters in the expression for the secondary nucleation rate of the \( \beta \) form were then estimated using a nonlinear optimization algorithm that minimizes the sum of squared residuals between the experimental and simulated solid phase compositions resulting from the previously described PBE model

\[
SSR = \sum_{i=1}^{N_e} \sum_{j=1}^{N_{d,i}} (w_{i,j}^{exp} - w_{i,j}^{sim})^2
\]  
(5.5)

where \( w^{exp} \) and \( w^{sim} \) are the experimental and simulated solid compositions expressed as weight percent \( \alpha \) polymorph, respectively. The experimental data of seeded transformations (runs 1 - 11) given in Table 5.2 has been utilized. The simplex approach was used through the \textit{fminsearch} function in MATLAB to minimize eq. (5.5) by varying the parameters in the nucleation rate expression of the \( \beta \) polymorph.

\textit{lsqnonlin} is generally faster than \textit{fminsearch} and its convergence behavior was found to be good for the estimation of the growth rate parameters. However, for the estimation of the nucleation rate parameters \textit{fminsearch} was used since the objective function is very shallow close to the optimum, and only \textit{fminsearch} was able to find the minimum.

Approximate confidence intervals of the estimated parameters were calculated as discussed in Section 3.3.
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

5.1.4 Results and discussion

In this section, first the growth rate of the $\beta$ polymorph is characterized based on seeded batch desupersaturation experiments. Then, the results of seeded polymorph transformation experiments are reported and the parameters in the expression for the secondary nucleation rate of the $\beta$ form are estimated. Finally, the model is used in a fully predictive manner to estimate the transformation times in unseeded polymorph transformation experiments.

Seeded batch desupersaturation experiments

The experimental method requires that only the added seed crystals grow during desupersaturation and that no new particles are formed. Therefore, FBRM was used to ensure that no significant nucleation occurred during the experiment. Nucleation can be detected by an increasing number of particle counts in the small size range. In none of the growth experiments nucleation was observed. The parameters in the growth rate expression were estimated as described above. The experimental conditions are given in Table 5.1. Two different sieve fractions were used to verify if a size-independent growth rate could be employed to describe the experimental data. The experimental results of the desupersaturation experiments are shown in Figure 5.3. The effect of different initial supersaturation can be observed in Figure 5.3(a) for the smaller seeds $F_{\beta,1}$ and in Figure 5.3(b) for the larger seeds $F_{\beta,2}$. It must be noted that for the $F_{\beta,1}$ seeds only half of the mass of seed crystals was used. Thus, owing to the larger surface area of the $F_{\beta,1}$ seeds, the depletion of the supersaturation is faster for this sieve fraction. However, experimental and simulated desupersaturation profiles are in reasonably good agreement and the growth rate parameters in eq. (5.3) were estimated
5.1 Calibration-based approach

Figure 5.3: Desupersaturation profiles for two different seed fractions $F_{\beta,1}$ (a) and $F_{\beta,2}$ (b) as given in Figure 5.1. Symbols: experimental data; Lines: simulation results.

as:

\[
\begin{align*}
    k_{G1} & = (5.7 \pm 0.1) \times 10^{-7} \text{ m s}^{-1} \quad (5.6) \\
    k_{G2} & = 0.95 \pm 0.01 \quad (5.7)
\end{align*}
\]

The crystal growth rates for the $\alpha$ and $\beta$ forms are plotted in Figure 5.4; the growth rates are expressed in terms of growth of a volume equivalent sphere in order to allow for direct comparison of the growth rates. The following equation was employed to calculate the growth rate $G$ of a volume equivalent sphere:

\[
G = G_L \left( \frac{6k_v}{\pi} \right)^{1/3}
\]

where $G_L$ is the growth rate of the characteristic dimension $L$, i.e. the length of the $\beta$ needle.
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

Figure 5.4: Comparison of the growth rates of the α and the β polymorph. Please note that the overall growth rate of a volume equivalent sphere is plotted for α and β, respectively.

It can be seen in Figure 5.4 that for $S_\alpha \geq 1.35$ the growth rate of α is larger than that of β, and vice versa. Thus, at supersaturations larger than 1.35 the formation of α over β is most likely kinetically favored in agreement with Ostwald’s rule of stages$^{90,91}$.

Seeded polymorph transformations - experimental

Seeded batch experiments were conducted to estimate the nucleation rate of the β polymorph. Moreover, the effect of different operating conditions, such as seed size, seed mass and stirring rate, was investigated. The operating conditions for the seeded polymorph transformation experiments are given in Table 5.2. Each experiment was carried out twice. The repeatability was found to be excellent, as shown by the very good agreement of the solid compositions and liquid phase concentrations in Figure 5.5 in the case of runs 1 and 2. In the following, the average of the two measured solid composition profiles, i.e. those corresponding
5.1 Calibration-based approach

to the repeated experiments, was taken to reduce the scattering in the measured solid compositions.

Figure 5.5: Experimental repeatability of seeded transformation experiments in terms of solid composition (a) and liquid phase concentration (b).

After addition of the $\alpha$ seed crystals to the saturated solution, the metastable $\alpha$ polymorph transforms slowly into the stable $\beta$ form as shown in Figure 5.5(a). Since nucleation and growth of the $\beta$ form are the rate limiting steps in the polymorph transformation of L-glutamic acid, the concentration stays at the solubility of the $\alpha$ polymorph until the last $\alpha$ crystal has dissolved, and the concentration eventually approaches the solubility of the $\beta$ form as can be seen in Figure 5.5(b).

The effect of initial seed mass on the duration of the transformation is shown in Figure 5.6 for the $F_{\alpha,1}$ sieve fractions, i.e. the seeds between 125 and 250 $\mu$m. It can be observed that with increasing mass of seed crystals the transformation time decreases. The results for the $F_{\alpha,2}$ and $F_{\alpha,3}$ sieve fractions, i.e. the seeds in the ranges of 250 - 350 and $> 350$ $\mu$m, are shown in Figure 5.7 and Figure 5.8, respectively. Also for these two sieve fractions the transformation time decreases with increasing seed mass. However, for the same seed mass the transformation time
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

Figure 5.6: Polymorph transformation with small seed crystals ($F_{\alpha,1}$ fraction). Symbols: experimental data; Lines: simulation results.

Figure 5.7: Polymorph transformation with medium seed crystals ($F_{\alpha,2}$ fraction). Symbols: experimental data; Lines: simulation results.
5.1 Calibration-based approach

Figure 5.8: Polymorph transformation with large seed crystals ($F_{\alpha,3}$ fraction). Symbols: experimental data; Lines: simulation results.

increases with decreasing seed size. This is somehow in contradiction to the previously proposed nucleation mechanism, i.e. surface nucleation on the $\alpha$ crystals\textsuperscript{58,92,93}, where a larger surface would provide more sources of nucleation. We believe that our $\alpha$ seed crystals were not pure in terms of polymorph composition. This had a large effect on the transformation and seemed to dominate the effect of a larger surface area. SEM micrographs of the $\alpha$ seeds are shown in Figure 5.9 and reveal the presence of $\beta$ crystals within the seeds. This inclusion has been reported previously\textsuperscript{93}, and it was suggested that $\beta$ nucleates on the surface of $\alpha$ and gets included in the $\alpha$ crystals by overgrowth owing to the higher growth rate of $\alpha$, which is expected based on the growth rates shown in Figure 5.4. Typically, this happens during the very early stages of the process, i.e. when supersaturation is high. Thus, it is very likely that the largest seed crystals have the highest density of these $\beta$ inclusions since they were formed at an earlier stage in the process. Unfortunately, it was not possible neither by Raman spectroscopy nor by powder X-ray diffraction to quantify the amount of $\beta$ inclusions in the seeds. Furthermore, we tested the lower detection limit of $\beta$ in the seeds. For this purpose
we added to the α seeds with the lowest contamination (fraction $F_{\alpha,1}$) a known amount of β crystals. It was found that below 5 wt.% no change in the Raman spectra could be observed. Nevertheless, in order to quantify the amount of β inclusion in the different sieve fractions we could estimate these parameters, i.e. the amount of contamination with β in the different α sieve fractions, together with the nucleation rate parameters.

Figure 5.9: SEM micrographs showing α seeds contaminated with β platelet particles.

It must be noted that the slope of the polymorph composition curve increases with increasing size of the seed crystals. For the $F_{\alpha,1}$ seeds the slope is almost zero at the beginning of the transformation and gets steeper for the $F_{\alpha,2}$ and $F_{\alpha,3}$ seeds. It is obvious that for the latter cases some β crystals must be present in the very beginning otherwise it would take much longer until the polymorph composition changes significantly.

The effect of stirring on transformation times is illustrated in Figure 5.10 and Figure 5.11 for two different sieve fractions. The transformation times decrease significantly with increasing stirring rates. In case we assume an integration limited growth mechanism of the β polymorph, which is highly justified at low supersaturations in a stirred vessel, the only mechanism being affected by the stirring rate and having an ef-
fect on the transformation time is the nucleation of the $\beta$ polymorph. Typically, attrition based secondary nucleation mechanisms show a dependency of the stirring rate. In the case the $\beta$ nucleation is induced by $\alpha$ surfaces, then the abrasion of $\alpha$ crystals will catalyze the nucleation of $\beta$ crystals as previously observed$^{92}$.

Figure 5.10: Effect of stirring on seeded polymorph transformations. Symbols: experimental data; Lines: simulation results.

Figure 5.11: Effect of stirring on seeded polymorph transformations. Symbols: experimental data; Lines: simulation results.
Seeded polymorph transformations - modeling

The parameter estimation technique presented in Section 5.1.3 was used together with the population balance model and the experimental results of the seeded transformation experiments to determine the nucleation rate of the \( \beta \) polymorph. To this aim runs 1 to 11 were employed for the parameter estimation and runs 12 to 15 were used to validate the obtained model. As discussed in the previous section no pure \( \alpha \) seeds could be produced, but the initial contamination with \( \beta \) could not be quantified experimentally. Therefore, the initial amount and characteristic size of \( \beta \) crystals in the seeds had to be estimated as well. It must be noted that simulations of the process without accounting for the initial contamination of the \( \alpha \) seeds did not give satisfactory results. In these simulations the polymorph composition with respect to \( \alpha \) was always overpredicted in the first half of the process, whereas it was underpredicted in the second. The overall shape of the polymorph composition curve could never be predicted correctly.

Based on these considerations, runs 1 to 11 were used to estimate seven parameters, namely those for the secondary nucleation of \( \beta \) on \( \alpha \) crystals, i.e. \( k_{J1}, k_{J2} \) and \( k_{J3} \) in eq. (5.2), the average size of the \( \beta \) crystals in the seeds, which is assumed to be the same for the three sieve fractions, and the percentage of \( \beta \) impurities in each sieve fraction. The nucleation rate parameters were estimated to be:

\[
\begin{align*}
    k_{J1} &= (7.6 \pm 0.2) \times 10^5 \text{ m}^{-3} \text{s}^{-1} \\
    k_{J2} &= 2.29 \pm 0.01 \\
    k_{J3} &= 0.88 \pm 0.01
\end{align*}
\]

While \( k_{J3} \) seems to be quite reasonable, i.e. in the range of 0.5 to 1, \( k_{J2} \)
appears to be rather large, though not unrealistic. For collisions with the impeller a value of 1 is expected and for particle-particle collisions a higher value of 2 can be derived. However, larger values than 2 have also been reported\textsuperscript{94}.

The initial amounts of the $\beta$ crystals included in the $\alpha$ seeds were estimated to be:

$$F_{\alpha,1} : w_{\beta,0} = 0.63 \pm 0.04 \text{ wt.\%}$$  \hspace{1cm} (5.12)
$$F_{\alpha,2} : w_{\beta,0} = 3.28 \pm 0.15 \text{ wt.\%}$$  \hspace{1cm} (5.13)
$$F_{\alpha,3} : w_{\beta,0} = 5.02 \pm 0.22 \text{ wt.\%}$$  \hspace{1cm} (5.14)

Finally, the particle size distribution of the $\beta$ crystals in the seeds was assumed to be the same for all three sieve fractions, namely a Gaussian distribution with a standard deviation of 20\% of the mean, since this gave reasonable PSDs as compared to measured PSDs of the $\beta$ form of L-glutamic acid, e.g. those given in Figure 5.1. The average size was estimated to be $L_{\beta,0} = 26.6 \pm 0.7 \mu\text{m}$, which seems to be in good agreement with the SEM images, e.g. those in Figure 5.9. It must be noted that at the beginning of the experiments, the experimental polymorph composition is always very close to 100 wt.\% of the $\alpha$ form. This is because, first, in the calibration model\textsuperscript{84} the seeds were assumed to be pure, and second, very low concentrations of $\beta$ could not be measured using Raman spectroscopy as mentioned above. It is worth noting that the presence of $\beta$ crystals in the seeds speeds up the transformation just because the new $\beta$ phase can start to grow immediately as the experiment starts, the faster the larger the value of $w_{\beta,0}$.

To validate the nucleation kinetics determined above, polymorph transformation experiments with the $F_{\alpha,3}$ seeds and different stirring rates were conducted. It must be noted that the model is fully predictive at
these conditions. The experimental data and the simulation results are shown in Figure 5.11. The transformation time decreases with increasing stirring rate and the trend is well predicted by the model. However, the agreement between model and experiment is less satisfactory than for the small seeds. The effect of attrition might be larger for the bigger seeds and hence the nucleation rate underpredicted.

In addition, we have tested whether the estimated contamination of the $\alpha$ seeds is reasonable. Therefore, we conducted transformation experiments with the $F_{\alpha,1}$ seeds and a seed concentration of 30 g/kg. To this we added a certain mass of crystals with a known particle size distribution, i.e. that corresponds to $F_{\beta,1}$ in Figure 5.1. Then, the transformation was monitored as described above. The results are shown in Figure 5.12. With increasing initial mass of the $\beta$ form, expressed as weight percentage $\alpha$ seed mass, the duration of the transformation decreases as expected. Moreover, the initial slope increases, thus showing the importance of $\beta$ contamination on the course of the transformation. The agreement between the modeling results and the experiments is reasonably good.

The growth and nucleation rates at $45^\circ$C of the two polymorphs of L-glutamic acid were determined previously based on unseeded batch experiments\textsuperscript{58}. The comparison of these rates with those being presented here shows some discrepancy. We believe that this is due to the fact that in the previous work nucleation and growth rates had been determined simultaneously, i.e. under conditions where they were highly correlated. Moreover, in that work the range of operating conditions was narrower than here and for instance the effect of stirring was not investigated. This work represents a step forward in that the different kinetics have been measured independently in order to develop models with higher accuracy and prediction quality.
Figure 5.12: Effect of initial amount of $\beta$ polymorph on transformation time. The initial concentration is given as mass percentage of the initial $\alpha$ seeds ($F_{\alpha,1}$ fraction with a concentration of 30 g/kg). Symbols: experimental data; Lines: simulation results.

**Unseeded polymorph transformations**

A series of unseeded transformation experiments has been performed at 45°C. In each of these experiments a monosodium glutamate solution was mixed with a hydrochloric acid solution to create the initial supersaturation. The initial supersaturation with respect to the $\alpha$ polymorph was varied from 1.75 to 3.75 by changing the initial concentrations. The stirring rate was kept constant at 300 rpm. A typical course of the solid phase composition and the solute concentration is shown in Figure 5.13(a).

The kinetic parameters as obtained from the seeded transformation experiments were used to simulate all the unseeded polymorphic transformations. The kinetic expressions and the estimated parameters are summarized in Table 5.3 and Table 5.4. In this case, the model accounts for growth and nucleation of both polymorphs, and is used in a fully predictive manner. The comparison between experiment and simulation
5. Monitoring and modeling the polymorph transformation of L-glutamic acid for one specific case is illustrated in Figure 5.13(a). From the simulation results induction time and endpoint were extracted and compared with experimental results. For further analysis, the total transformation period, i.e. induction time plus actual transformation period indicated as $t_i$ and $t_p$ in Figure 5.13(a), respectively, was considered and plotted in Figure 5.13(b). The lowest supersaturation as applied in the conducted induction time experiments equaled 2.0, hence the simulation results shown as dashed line are based on extrapolated nucleation kinetics.

<table>
<thead>
<tr>
<th>mechanism</th>
<th>expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ nucleation, $J_\beta$</td>
<td>$J_\beta = 7.6 \times 10^5 m_\alpha^{2.29} c^{0.88}$</td>
</tr>
<tr>
<td>$\beta$ growth, $G_\beta$</td>
<td>$G_\beta = 5.7 \times 10^{-7} (S_\beta - 1)^{5/6} \exp(-0.95 S_\beta)$</td>
</tr>
</tbody>
</table>

Table 5.4: Elucidated mechanisms and their kinetics expressions at 45°C used in the population balance model.

In the simulations in Figure 5.13(b), the total transformation time decreases for an increasing initial supersaturation. The experiments exhibit a similar trend, but there is a significant amount of scattering. The displayed experimental points are the result of at least three transformation experiments and the variance is given as error bars in the plot.

The large variability in the experimental data can be explained based on an analysis of an unseeded transformation experiment. After the formation of $\alpha$ crystals and the depletion of the supersaturation, an unseeded polymorph transformation is governed by the same phenomena as a seeded transformation experiment, i.e. dissolution of the $\alpha$ polymorph and nucleation and growth of the $\beta$ polymorph. These phenomena are well reproducible as shown through the series of seeded transformation experiments as discussed in this work, therefore the scattering in Figure 5.13(b) is most likely due to primary nucleation of $\alpha$ and $\beta$ crystals during the induction period. Primary nucleation as a stochastic
Figure 5.13: (a) A representative unseeded transformation experiment with initial supersaturation $S_\alpha = 2.5$ where $t_i$ and $t_p$ represent induction time and transformation period, respectively. Lines represent simulation results and symbols experimental data. (b) Total transformation period ($t_i + t_p$), experimental (symbols) and simulated (line), as a function of the initial supersaturation. Experimental transformation times are the result of at least three transformation experiments and the variance is given as error bars in the plot.

phenomenon is known to be difficult to reproduce and experiments conducted with great care may still exhibit a large variability\textsuperscript{79,87,95}. During the induction period, the $\alpha$ as well as the $\beta$ polymorph nucleate simultaneously, both with a certain variability. As discussed above and shown in Figure 5.12, small variations in the relative amount of the $\beta$ form as well as in the obtained particle size distribution have a significant effect on the transformation times and most likely result in the observed scattering in Figure 5.13(b).
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

5.1.5 Conclusions

The quantitative use of in situ Raman spectroscopy enables new insights in the fundamental mechanisms of polymorph transformations. The solvent mediated polymorph transformation of L-glutamic acid is governed by nucleation, growth and dissolution of the metastable $\alpha$ polymorph and the nucleation and growth of the stable $\beta$ polymorph. While the first three kinetics were determined previously,$^{52,58,87}$ the latter are measured in this study. We used in situ Raman spectroscopy to determine the secondary nucleation rate of the stable $\beta$ polymorph of L-glutamic acid at various operating conditions. The proposed method is based on seeded transformation experiments and requires that the growth rate of the $\beta$ polymorph is measured independently. Following the method proposed earlier,$^{52}$ we have measured the growth rate of the $\beta$ form on the basis of separate seeded batch desupersaturation experiments. Then, the data of the polymorph transformation experiments can be used together with a population balance model and a nonlinear least-squares optimization routine to estimate the secondary nucleation rate of the $\beta$ polymorph, which is otherwise not directly accessible. From the experimental observations it was concluded that an attrition-based nucleation mechanism governs the transformation of the metastable $\alpha$ to the stable $\beta$ polymorph. To the best of our knowledge, for the first time, the effect of stirring on the course of the polymorph transformation was modeled using a population balance model and implementing the relevant kinetics. Finally, the proposed model allows calculating the course of a polymorph transformation in a fully predictive manner for seeded as well as for unseeded conditions under a wide range of operating conditions.
5.2 Calibration-free approach

5.2.1 Introduction

During the last decades, the number of publications that report utilization of in situ characterization tools to monitor a wide variety of chemical processes has increased significantly. Such process analytical technologies (PATs) include various spectroscopic techniques, among them infrared and Raman spectroscopy. The vast majority of the in situ spectrometers record the data through fiber-optic technology, enabling rapid measurement of spectra over a large spectral range, and resulting in a significant increase in the amount of data obtained per experiment. In order to handle the large amount of data and to extract accurate and robust information about a particular process, multivariate methods have been applied more and more frequently. Those multivariate methods include principal components regression (PCR) and partial least squares regression (PLSR), which both reduce the dimension of the regression problem by introducing a small number of latent variables that are linear combinations of the original variables. Although providing highly accurate estimations of process variables such as solute concentration and solid phase composition, multivariate methods require a significant amount of calibration work. Besides, changes in the performance of the instrument, e.g. a decrease in laser power, can seriously limit or might even invalidate the calibration model. Using the solute concentration and the solid phase composition resulting from a multivariate analysis of the experimental data, a wide variety of physical and chemical phenomena can however be modeled, i.e. the kinetic expressions can be determined and the corresponding parameters can be estimated. Ultimately, accurate kinetic information allows process modeling and performance prediction and enables process design, optimization and control.
While the multivariate calibration of for instance mid-infrared spectroscopic data is extensively reported by several groups\textsuperscript{15–17,67,84}, the multivariate analysis of Raman data has been discussed less frequently and is known to be challenging due to influences of many process parameters, e.g. particle size and shape\textsuperscript{22,23,62,64,65,84}. The extensive amount of calibration work required (several weeks or even months) slows down and hampers the application of in situ Raman spectroscopy, in academia as well as in industry.

Recently, it has been shown using ultraviolet-visible\textsuperscript{54,96,97}, near-infrared\textsuperscript{54,97,98}, and mid-infrared\textsuperscript{54,97,99–101} spectroscopy that kinetic parameters can be obtained directly by fitting the time-resolved spectroscopic measurements. This method is called multivariate kinetic modeling and has been applied to several homogeneous chemical reactions thus estimating the corresponding parameters describing reaction kinetics. This approach requires a model that describes the course of the concentrations of the different analytes over time. In the case of chemical reactions occurring in a homogeneous stirred tank, such a model is a set of coupled ordinary differential equations (ODEs) combined with mass and energy balances. Together with an optimization routine, such a model can be used to estimate the unknown kinetic parameters by fitting the measured spectra directly, thereby making calibration unnecessary.

Here, a similar approach has been applied to estimate kinetic parameters governing a solvent-mediated polymorph transformation, i.e. that of the metastable $\alpha$ form of L-glutamic acid into the stable $\beta$ form\textsuperscript{77}. It is worth noting that there are two major novelties with respect to previous work by other authors. The first is the application of the modeling approach to a heterogeneous batch process, which results in a significantly more complex mathematical description. The second is the application of in
5.2 Calibration-free approach

situ Raman spectroscopy in the case where the signals resulting from the liquid as well as from the solid phase are used to model the process.

This work complements two recent papers of ours. In the first\textsuperscript{84}, the multivariate calibration of Raman data is discussed and it is shown that the Raman signal depends not only on the solid concentration of the two polymorphs considered but also on their particle size, which changes in time during crystallization. A linear relationship between the Raman spectra and the solid phase composition in the case of L-glutamic acid’s solvent-mediated polymorph transformation was assumed; an extensive calibration set consisting of several particle populations of different composition and different average size was used together with proper pre-processing techniques, thus showing that in situ Raman spectroscopy can indeed be applied in a quantitative manner. The second paper\textsuperscript{77} describes the modeling of the polymorph transformation of L-glutamic acid using the solid phase composition profiles resulting from the calibration discussed previously. This sequence of calibration followed by fitting the resulting solid phase compositions can be considered as the classical approach to obtain process parameters governing crystallization processes.

This study presents an alternative approach to reach the same objective, i.e. to characterize the kinetics of growth and nucleation mechanisms in a suspension during for instance a polymorph transformation. The kinetic parameters are estimated through fitting the Raman data directly. It is assumed that the Raman spectra measured in situ scale linearly with the amount of scattering material per unit volume, likewise in absorbance spectroscopy, e.g. infrared spectroscopy. This feature is demonstrated in Figure 5.14 where the Raman signal intensity depends linearly on the solute concentration as well as on the concentration of solid in the case of suspensions. To assess this novel approach, the estimated parameters are
then compared to those obtained by fitting the solid phase composition profiles that resulted from the multivariate calibration. This approach avoids any calibration and knowing that the calibration of Raman data is a lengthy procedure, it facilitates the otherwise time-consuming estimation of the kinetic parameters governing a solid-state transformation. The previous paper and this work both approximate the relationship between the measured Raman spectra and the process variables and the estimated concentration profiles can until now not be verified quantitatively using for instance HPLC as it is done in the case of homogeneous reactions.

5.2.2 Modeling the Raman data

In this work, the time-resolved Raman spectra were used in a calibration-free manner. A detailed discussion about the notation and multivariate
5.2 Calibration-free approach

calibration-free analysis of spectroscopic data can be found in Section 3.1 and Section 3.1.2, respectively.

As discussed in the introduction we also assume that the Raman spectra measured in situ scale linearly with the amount of scattering material per unit volume. The measured signal intensity at a certain Raman shift $\lambda$ and at a given time $t$, $x(t, \lambda)$, can then be expressed as a linear combination of the signals corresponding to each analyte, i.e.:

$$x(t, \lambda) = \sum_{l=1}^{d} c_l(t) a_l(\lambda) + e(t, \lambda) = \hat{x}(t, \lambda) + e(t, \lambda) \quad (5.15)$$

where $a_l(\lambda)$ represents the intensity at Raman shift, $\lambda$, of the pure-analyte spectrum corresponding to the $l^{th}$ analyte, $c_l(t)$ denotes the concentration of the $l^{th}$ analyte at time $t$, and $e(t, \lambda)$ represents the experimental error, i.e. the noise and the nonidealities of the measurement. By discretizing the spectral and time coordinates, the spectral matrix $X$ ($n \times m$) can then be written in matrix notation as:

$$X = CA + E = \hat{X} + E. \quad (5.16)$$

The element $x_{ij}$ of the matrix $X$ represents the $j^{th}$ measured intensity for the $i^{th}$ sample and $C$ ($n \times d$) represents the state matrix, i.e. the concentrations of the $d$ analytes involved, where the $l^{th}$ column of $C$ is the concentration profile in time of the $l^{th}$ analyte. The $l^{th}$ row in the matrix $A$ ($d \times m$) denotes the discretized pure-analyte spectrum for analyte $l$; the matrix $E$ ($n \times m$) is the matrix of experimental errors.

It is worth noting that the discretization of the Raman shift coordinate as well as the sampling in time are determined by the settings of the instrument, i.e. the Raman spectrometer.

The key assumption is that the process can be described by a model
that predicts how the concentrations of the different analytes evolve in time, i.e. it provides a modeled time-resolved concentration matrix \( \hat{C}(k) \), where the vector \( k \) consists of \( p \) model parameters that are physicochemical or transport properties of the system. The classical approach is to develop a calibration model \( B \) and to use this calibration to calculate the concentration matrix \( C = XB \) from the measured spectral matrix \( X \). One can then estimate the model parameters \( k \) by minimizing in some sense the difference between the matrices \( \hat{C}(k) \) and \( C \). This classical approach relies on a calibration procedure, which is often very time-consuming.

One can however also use the time-resolved spectra, i.e. the matrix \( X \) itself, to estimate directly the model parameters without converting explicitly the spectral matrix \( X \) into the measured concentrations \( C \). Formally, this would imply minimizing for instance the sum \( S_r \) of the squares of the elements of the residual matrix \( R(k) \) \((n \times m)\):

\[
R(k) = X - \hat{C}(k)A
\]  

(5.17)

where both the \( p \) elements of the vector \( k \) and the \( d \times m \) elements of the matrix \( A \) are unknown and should be obtained through some optimization procedure, i.e. a rather challenging task considering that the number of elements of \( A \) can be very large. However, using the pseudoinverse matrix of \( \hat{C}(k) \), i.e., \( \hat{C}^+(k) = (\hat{C}^T(k)\hat{C}(k))^{-1}\hat{C}^T(k) \), the expression can be rewritten as:

\[
R(k) = X - \hat{C}(k)\hat{C}^+(k)X = [I - \hat{C}(k)\hat{C}^+(k)]X.
\]  

(5.18)

By minimizing the sum of the squares of the elements of the residual matrix \( R(k) \), \( S_r \), one obtains the \( p \) unknown model parameters, i.e. a much easier task than solving eq. (5.17)\(^{39,41,54} \).
5.2.3 Modeling the polymorph transformation

The mathematical model of the transformation process is based on population balance equations (PBEs). Using proper nucleation, growth and dissolution rate expressions, this model allows for the calculation of the solute concentration, solid phase concentrations and particle size distributions of both polymorphs, thus providing the time-resolved information that constitutes the columns of the state matrix $\hat{C}(k)$. The model equations are the same as in Section 5.1.3 and are solved using the method of moments as described in Section 3.2.2. This approach was applied because it required less computation time and the only interest was to calculate the time-evolution of the liquid and solid concentration and not the particle size distribution itself. In the case of unseeded polymorph transformations, the particle size distribution of the $\alpha$ form, required to calculate its dissolution rate, was reconstructed using the method of fitting splines and more details can be found elsewhere.

The nucleation, growth and dissolution kinetics of the $\alpha$ polymorph and the nucleation and growth kinetics of the $\beta$ polymorph were determined and discussed in detail in the first part of this chapter. These kinetics were used in this work as reported in Table 5.5.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Kinetic expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ nucleation, $J_\alpha$</td>
<td>$2.4 \times 10^8 S_\alpha \exp(-\frac{4.4}{\ln^2 S_\alpha})$</td>
</tr>
<tr>
<td>$\alpha$ growth, $G_\alpha$</td>
<td>$9.6 \times 10^{-7}(S_\alpha - 1)^{2/3}(\ln S_\alpha)^{1/6} \exp(-\frac{0.54}{\ln S_\alpha})$</td>
</tr>
<tr>
<td>$\alpha$ dissolution, $D_\alpha$</td>
<td>$2.2 \times 10^{-4}(S_\alpha - 1)$</td>
</tr>
<tr>
<td>$\beta$ nucleation, $J_\beta$</td>
<td>$k_1 m_\alpha^k$</td>
</tr>
<tr>
<td>$\beta$ growth, $G_\beta$</td>
<td>$5.7 \times 10^{-7}(S_\beta - 1)^{5/6} \exp(-\frac{0.95}{S_\beta - 1})$</td>
</tr>
</tbody>
</table>

Table 5.5: Mechanisms and their kinetics expressions at $45^\circ$C used in the detailed process model. A detailed discussion about the selection of the expressions and their parameters can be founded in the first part of this chapter.
In order to demonstrate the applicability of fitting multivariate Raman data, the parameters in the secondary nucleation rate expression were assumed to be unknown and were estimated based on a set of seeded polymorph transformation experiments. The secondary nucleation rate expression of the $\beta$ polymorph reads as follows:

$$J_\beta = k'_1 m_\alpha^{k_2}$$

(5.19)

where $m_\alpha$ represents the concentration of the $\alpha$ polymorph in g solid/kg solvent. For the sake of numerical stability, the pre-exponential factor has been written as:

$$k'_1 = e^{5k_1}.$$

(5.20)

From this expression it can easily be seen that the parameters $k_1$ and $k_2$ are strongly correlated, i.e. for a constant mass $m_\alpha$ combinations of $k_1$ and $k_2$ that lay on the line $5k_1 + k_2 \ln m_\alpha = constant$ result in the same secondary nucleation rate $J_\beta$.

Additionally, it has been demonstrated for seeded transformation experiments that the presence of a small amount of $\beta$ crystals in the seed crystals influences the overall duration of a polymorph transformation significantly\textsuperscript{77}. The concentration of the $\beta$ impurities can be defined as an additional unknown parameter, i.e. $w_{\beta,i}$ where $i$ represents a specific fraction of seed crystals, and can be estimated together with the nucleation rate parameters. The average size of the $\beta$ crystals in the $\alpha$ seeds was assumed to be constant and to be equal to 27 $\mu$m and a Gaussian distribution with a standard deviation of 20% of the mean particle size was assumed as particle size distribution for the initial $\beta$ form crystals\textsuperscript{77}. 

104
5.2 Calibration-free approach

5.2.4 Results and discussion

In this section, the possibility of fitting time-resolved Raman data is thoroughly investigated using synthetic as well as experimental data of the solvent-mediated polymorph transformation of L-glutamic acid. The concept is to simulate the process, i.e. to calculate the state matrix \( \hat{C}(k) \) using some initial parameters, and together with the spectral matrix \( X \) the kinetic parameters governing the transformation are estimated iteratively by fitting the time-resolved Raman spectra, i.e. by minimizing the residuals in eq. (5.18). In the first part synthetic Raman data is generated through simulations and in the second part experimental Raman data is used.

Simulated Raman data

Unseeded and seeded transformations were simulated using the population balance model described above combined with the previously estimated kinetics as given in Table 5.5.

In order to simulate Raman data, i.e. to calculate a synthetic spectral matrix \( \hat{X} \), pure-analyte spectra have to be used. Here, single Gaussian peaks were assumed as pure-analyte spectra for the solute, the \( \alpha \) and \( \beta \) polymorph. Once the concentration matrix \( \hat{C} \) is known, the spectral matrix \( \hat{X} \) (\( \hat{X} = \hat{C}A \)) can be calculated using eq. (5.16) and white noise with a standard deviation of 1500 a.u. was added to \( \hat{X} \) in order to generate realistic Raman spectra.

As discussed in Section 3.3, standard optimization algorithms are in principle able to solve the optimization problem associated to eq. (5.18) and different methods were applied and compared in this work. Given the fact that there are two unknown parameters only, one can discretize
the parameter space and perform simulations for many combinations of \( k_1 \) and \( k_2 \) and plot the sum of squared residuals \( S_r \) as a function of \( k_1 \) and \( k_2 \). This has been done for the unseeded as well as for the seeded transformations in order to gain insight in the optimization problem; the related figures can be found elsewhere\(^{49}\).

To obtain robust parameters and to reduce possible correlation between the unknown parameters, optimizations were performed using multiple experiments under different operating conditions\(^{54}\). For the seeded transformations, three different seed masses were employed and for the unseeded transformations three different initial supersaturations were used. In both cases, this change in operating conditions led to different levels of the suspension density.

**Unseeded polymorph transformation**

Three simulations of unseeded transformations at 45\(^\circ\)C at different initial supersaturations, namely \( S_\alpha = 2.0, 2.25, 2.75 \), were performed with \( k_1 = 1.8 \) and \( k_2 = 2.3 \). The concentration profiles of the solute, the \( \alpha \) and the \( \beta \) polymorph, are shown in Figure 5.15(a) for \( S_\alpha = 2.25 \). The time-resolved Raman spectra obtained using the assumed pure-analyte Raman spectra are shown in Figure 5.15(b).

Starting from the simulated Raman spectra, it is now attempted to recover the parameter values initially selected. Different optimization techniques were applied to estimate these parameters, among them the simplex method, the Newton-Gauss-Levenberg/Marquardt (NGL/M) method and simulated annealing. The three different methods resulted all in the correct parameters (exactly correct to two significant figures), and the simplex method was found to be most efficient in terms of computational effort and to be most robust. The sum of squared
Figure 5.15: Simulated concentrations, matrix \( \hat{C} \), (a) and the resulting simulated time-resolved Raman spectra (b) for an unseeded transformation with \( S_\alpha = 2.25 \) at 45°C.

residuals \( S_r \) as a function of \( k_1 \) and \( k_2 \) can be found elsewhere\(^{49}\) and the lowest value for \( S_r \) is indeed located at the values of the initially given parameters and the parameters exhibit a strong correlation.

Seeded polymorph transformation

Three simulations of seeded polymorph transformations at 45°C using different amounts of seed crystals, namely \( m_{seeds} = 15, 30, 50 \) g/kg, were performed with \( k_1 = 1.9 \) and \( k_2 = 2.4 \) and assuming pure seed crystals, i.e. \( w_\beta = 0 \).

Starting from the simulated time-resolved Raman data, the initially given parameters were again recovered using different optimization techniques. The three different methods resulted all in the correct parameters (exactly correct to three significant figures) and, as in the case of unseeded transformations, the simplex method was found to be fastest and
most robust. The sum of squared residuals as a function of $k_1$ and $k_2$ can be found elsewhere\textsuperscript{49}. The minimum is located in a shallow valley and the parameters exhibit a strong correlation.

**Experimental Raman data**

In this section, the method of fitting time-resolved Raman data is applied to a series of seeded transformation experiments monitored using in situ Raman spectroscopy. The seeded transformations were performed using small (mean particle size $d_{43} = 188 \, \mu m$), intermediate ($d_{43} = 303 \, \mu m$) and large ($d_{43} = 412 \, \mu m$) $\alpha$ crystals as seeds. The details about the preparation of the seed crystals and the particle size distributions can be found elsewhere\textsuperscript{77,84}. The initial suspension density was 15, 30 and 50 g/kg. The time-resolved Raman spectra ($835 \leq \lambda \leq 885 \, \text{cm}^{-1}$) of the seeded transformation experiments, corrected for an offset and normalized, were fitted directly using the detailed process model and the unknown parameters are those that appear in the expression for the secondary nucleation rate of the $\beta$ form as in eqs. (5.19) and (5.20). The dissolution rate of the $\alpha$ polymorph and the growth rate of the $\beta$ form have been used as reported in Table 5.5.

The material and the experimental equipment that was used to obtain the reported results have been described earlier\textsuperscript{77,84}.

**Fitting time-resolved Raman data**

To illustrate the concept of fitting time-resolved Raman data, two of the seeded transformation experiments, i.e. using 15 and 50 g/kg of the small seed crystals, are now analyzed in more detail. It should be noted that both experiments are analyzed independently. The left hand
5.2 Calibration-free approach

side (parts a, c and e) and the right hand side (parts b, d and f) of Figure 5.16 correspond to the transformation experiments using 15 and 50 g/kg of the small seed crystals, respectively. The parts a and b show the measured time-resolved Raman spectra, i.e. the matrix $X$ corrected for an offset and normalized. The higher suspension density results in a higher signal to noise ratio when comparing the parts a and b. The time-resolved Raman spectra are then fitted as discussed in Section 5.2.2 and the parts c and d display the time evolution of the intensities at different Raman shifts, i.e. several columns of the matrix $X$ whose characteristic Raman shifts are indicated in parts a and b, together with the modeled intensities at the same Raman shifts. As it can be seen, the time-resolved Raman data is fitted satisfactorily in both cases. The fitting of the time-resolved Raman data results in estimations of the kinetic parameters that provide the modeled analyte concentrations, i.e. the state matrix $\hat{C}$, which are shown in parts e and f.

Comparison to classical approach using calibration

The multivariate calibration described previously\textsuperscript{84} enables the comparison between the modeled concentrations obtained by fitting the time-resolved Raman spectra and the concentrations estimated using this calibration model. Figures 5.17(a) and 5.17(b) show this comparison for the seeded transformation experiments using 15 and 50 g/kg of the small sieve fraction, respectively. The agreement between the modeled (lines) and estimated (symbols) concentration profiles is good and demonstrates that accurate concentration profiles can be obtained by fitting time-resolved Raman data.

In previous work\textsuperscript{77}, three different sieve fractions were used to model the solid-state transformation of L-glutamic acid, i.e. unknown model pa-
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

Figure 5.16: Fitting multivariate Raman data using 15 (parts a, c and e) and 50 (parts b, d and f) g/kg of the small seeds. The measured spectra acquired during the seeded transformations are shown in parts a and b. Parts c and d plot the measured and the fitted intensities at different Raman shifts. The modeled concentration profiles of the α and the β form and the solute based on fitting the time-resolved Raman spectra directly are displayed in parts e and f.
5.2 Calibration-free approach

Figure 5.17: Comparison between the modeled concentration profiles based on fitting the time-resolved Raman spectra (lines) and the concentrations estimated using the multivariate calibration for 15 (a) and 50 (b) g/kg of the small sieve fraction. The solid phase composition profiles (symbols) and the predictions based on fitting the time-resolved Raman spectra (solid lines) and the predictions based on fitting the estimated solid phase composition profiles (dashed lines) in the case of the small (c) and the intermediate (d) sieve fraction using 15, 30 and 50 g/kg of seed crystals.
parameters were estimated by fitting the measured solid phase composition profiles. A comparison can be made between process models obtained by fitting the time-resolved Raman data and obtained by fitting the solid phase composition profiles for these three sieve fractions. Figure 5.17 shows the measured (symbols) and the modeled (lines) solid phase composition profiles for the small seeds in part c and for the intermediate seeds in part d using three different seed masses. The two different lines styles represent the two different modeling approaches, i.e. the modeling results based on fitting the time-resolved Raman spectra (solid lines) and the modeling results based on fitting the solid phase composition profiles (dashed lines). Clearly, the fits are in all cases satisfactory and the differences between the two modeling approaches are small.

Table 5.6 summarizes the optimization results for the different series of seeded transformation experiments, i.e. using the small, intermediate and large sieve fractions. It is worth noting that three optimization problems, each consisting of three experiments using different seed masses, were analyzed. As it can be seen, the estimated parameters based on the two approaches, i.e. fitting the Raman spectra and fitting the estimated solid phase compositions, are similar in all cases. The confidence intervals for the parameters estimated directly from Raman spectra are significantly smaller as compared to the confidence intervals estimated using the solid phase compositions.

To make a full comparison to the kinetic parameters given previously, all experiments using different sieve fractions as seed crystals have been analyzed together using their time-resolved Raman data to fit the model parameters. This yielded the following values of the two kinetic param-
### 5.2 Calibration-free approach

<table>
<thead>
<tr>
<th></th>
<th>Raman spectra</th>
<th>Solid composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>$k_1$</td>
<td>2.002 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>$k_2$</td>
<td>2.218 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>$w_{β,1}$</td>
<td>0.625 ± 0.033</td>
</tr>
<tr>
<td>Intermediates</td>
<td>$k_1$</td>
<td>2.049 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>$k_2$</td>
<td>2.213 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>$w_{β,2}$</td>
<td>2.680 ± 0.024</td>
</tr>
<tr>
<td>Large</td>
<td>$k_1$</td>
<td>0.984 ± 0.014</td>
</tr>
<tr>
<td></td>
<td>$k_2$</td>
<td>3.700 ± 0.027</td>
</tr>
<tr>
<td></td>
<td>$w_{β,3}$</td>
<td>5.448 ± 0.032</td>
</tr>
</tbody>
</table>

Table 5.6: Optimization results based on fitting the time-resolved Raman spectra compared to fitting the solid phase composition profiles using the three different sieve fractions. Parameters $k_1$ and $k_2$ constitute the secondary nucleation rate expression, $w_{β,i}$ represents the initial amount of impurities of $β$ in the seed crystals expressed as wt.% of total seed mass. All optimizations were performed using the simplex algorithm.

It should be emphasized that the estimated parameters are not identical to the ones given previously\textsuperscript{77}, i.e. $k_1 = 1.93$, $k_2 = 2.29$ as the secondary nucleation rate parameters, $w_{β,1} = 0.63$, $w_{β,2} = 3.28$ and $w_{β,3} = 5.02$ as the impurity content for the small, the intermediate and the large sieve fractions, respectively. One possible explanation for this discrepancy is the known correlation between the secondary nucleation rate parameters and of the three amounts of $β$ crystals in the seeds:

\begin{align*}
  k_1 &= 1.82 ± 0.01 \\   
  k_2 &= 2.44 ± 0.01 \\   
  w_{β,1} &= 0.74 ± 0.03 \\   
  w_{β,2} &= 3.13 ± 0.12 \\   
  w_{β,3} &= 5.54 ± 0.20 
\end{align*}

(5.21) (5.22) (5.23) (5.24) (5.25)
5. Monitoring and modeling the polymorph transformation of L-glutamic acid

ters, i.e. different combinations of the parameters result in essentially the same nucleation rate and a second is the fact that the developed multivariate calibration model might not be perfect and the resulting solid composition profiles might deviate slightly from the actual ones. Additionally, the scattering of the measured Raman spectra is different than the scattering of the measured concentrations which might also shift the global minimum and therefore yield slightly different parameters.

It should be emphasized that the objective of this work is not to provide the most accurate detailed process model for this polymorph transformation, but to demonstrate that very similar models can be obtained by fitting the time-resolved Raman spectra directly and by fitting the measured solid phase composition profiles thereby omitting extensive calibration efforts.

As shown in the first part of this chapter, the repeatability of unseeded transformation experiments using L-glutamic acid was found to be poor, hence no unseeded transformations were analyzed to demonstrate the applicability of this approach.

5.2.5 Conclusions

In this study a novel approach to apply in situ Raman spectroscopy in a quantitative manner, avoiding the development of a calibration model, is presented. This approach fits the measured time-resolved Raman spectra directly and results in the concentrations of the different analytes as a function of time. The method has been discussed and tested thoroughly using synthetic as well as experimental Raman data acquired during the solvent-mediated polymorph transformation of L-glutamic acid. Using data generated through simulations it has been shown that
5.2 Calibration-free approach

the initially given parameters can be estimated through fitting the time-
resolved Raman spectra. Using experimental Raman spectra it has been
demonstrated that the modeled concentration profiles based on fitting
the time-resolved Raman data show a good agreement with the esti-
mated concentrations using a multivariate calibration model. Addition-
ally, the estimated kinetic parameters based on fitting Raman spectra
are very similar to those obtained through fitting the solid phase com-
position profiles obtained from the calibration model. Therefore, this
approach enables quantitative understanding of a solid-state transform-
lation process without the necessity of developing a calibration model
and thereby saving a significant amount of time and resources. Several
solid-state transformations have been monitored quantitatively using in
situ Raman spectroscopy and certainly many more will be in the future,
therefore the applicability of this approach is expected to be broad.
Chapter 6

Estimating growth rates calibration-free using in situ spectroscopy

Seeded batch desupersaturation experiments of paracetamol in water were monitored using in situ ATR-FTIR and in situ Raman spectroscopy. Based on the linear relationship between the measured signal and the concentrations of the involved components, in situ ATR-FTIR and Raman spectroscopy could be used quantitatively in a calibration-free manner to estimate the crystal growth rate of paracetamol. Using a population balance model and a nonlinear least-squares optimization routine, it was shown that by fitting the measured time-resolved ATR-FTIR spec-

6.1 Introduction

Crystal growth is one of the fundamental mechanisms that govern crystallization processes and several approaches can be used to estimate its rate\(^1\). One popular approach to determine the overall crystal growth rate is to add an amount of seed crystals with known particle size distribution to a solution that is supersaturated and to measure the depletion of the supersaturation after the seed crystals are added. The crystal growth rate is then estimated using a process model that predicts the evolution of the supersaturation over time, which can then be fitted to the measured supersaturation values by changing the parameters that appear in the growth rate expression. There are several ways to measure these concentration values, e.g. by taking samples during the experiment and to determine their concentrations gravimetrically\(^{102}\), by using densitometry\(^{103}\) or by using in situ ATR-FTIR spectroscopy\(^{52,77}\).

Recently, we proposed a calibration-free approach to apply in situ Raman spectroscopy quantitatively in the case of a polymorph transformation\(^{49}\). It was demonstrated that the kinetic parameters can be obtained by fitting the measured time-resolved Raman spectra directly, thereby
 Estimating growth rates calibration-free using in situ spectroscopy

avoiding extensive calibration efforts. This approach is based on the assumption that the measured signal scales linearly with the concentration of the involved components and has been applied previously to study the kinetics of homogeneous reactions \textsuperscript{39,54}.

In this work, we address the application of in situ ATR-FTIR and Raman spectroscopy to estimate the crystal growth rate of paracetamol in water. The growth rate parameters can be determined through fitting the evolution of the supersaturation values measured using ATR-FTIR spectroscopy over time during seeded batch crystallization experiments, i.e. a robust and reliable approach as shown previously \textsuperscript{52,77}. It is well-known however that the measured infrared absorbance scales linearly with the solute concentration, as shown in the case of paracetamol in water in Figure 6.1(a). Figure 6.1(b) demonstrates that also the measured Raman signal depends linearly on the concentration of solid as well as on the solute concentration in the case of saturated suspensions and solutions, respectively. Based on these observations, the calibration-free approach is expected to be effective also in the case of the estimation of crystal growth rate parameters using at the same time in situ ATR-FTIR and in situ Raman spectroscopy.

It should be emphasized that there are two major novelties with respect to previous work by other authors and by ourselves. The first is the calibration-free application of in situ ATR-FTIR spectroscopy to determine crystal growth rate parameters, thereby avoiding time-consuming calibration. The second is the use of in situ Raman spectroscopy to estimate these growth rate parameters in a calibration-free manner. It is observed that crystals attach to ATR probe windows much more often than they do to in situ Raman probes, thus the latter result promises to have a major impact in the characterization of kinetic phenomena in crystallization processes. Finally, this work demonstrates the broad
applicability of the approach to use in situ spectroscopic techniques in a calibration-free manner that was presented recently\textsuperscript{49}.

Figure 6.1: The integrated peak area (1200 – 1290 cm\textsuperscript{-1}) of paracetamol solutions in the case of ATR-FTIR spectroscopy (a). The integrated peak area in the case of paracetamol solutions (filled symbols, 1155 – 1185 cm\textsuperscript{-1}) and in the case of saturated suspensions (open symbols, 780 – 810 cm\textsuperscript{-1}) measured with Raman spectroscopy (b). All measurements were performed at 20°C and the lines are a guide for the eye.

### 6.2 Materials and methods

#### 6.2.1 Materials

In this work, paracetamol or 4-acetamidophenol (98%, Sigma-Aldrich, Buchs, Switzerland) and deionized water were used in all experiments. Paracetamol has three known polymorphs and the transformation from one form into another has been described elsewhere.\textsuperscript{104} In this work, the thermodynamically stable octahedral form I has been used and no indications of a polymorph transformation have been observed, i.e. significant changes in the measured time-resolved Raman spectra have not
been observed in any of the reported experiments. The seed crystals were prepared by precipitation from an ethanol solution at 5°C and different fractions were obtained by wet sieving of the obtained crystals. The sieve fraction from 125 to 250 µm was used for further experiments and its particle size distribution is shown in Figure 6.2.

![Particle size distribution](image)

Figure 6.2: The measured particle size distribution of the seed crystals that were used in all batch desupersaturation experiments. The line indicates the fit of the measured points that was used in the population balance model and the average particle size $d_{43}$ equalled 156 µm.

### 6.2.2 Experimental setup

All experiments were performed in a jacketed glass reactor of 500 mL volume. The vessel was temperature controlled using a Minstat 230-3 thermostat and a Pt 100 temperature sensor and was equipped with a four-blade glass impeller (LTS, Biel-Benken, Switzerland). All probes were immersed, and ATR-FTIR, Raman and FBRM measurements could be performed simultaneously. The experimental setup was described in detail earlier.\textsuperscript{84}
6.2 Materials and methods

6.2.3 Characterization techniques

The details about in situ and offline characterization techniques used are described elsewhere\(^8^4\). The in situ measurements, i.e. ATR-FTIR, Raman and FBRM, were performed over a 60 seconds interval. The multivariate calibration procedure of the ATR-FTIR is discussed in detail elsewhere\(^8^4\) and has been successfully applied in other works\(^7^7,^1^0^5,^1^0^6\). This multivariate calibration routine enables accurate in situ measurement of the solute concentration as validated offline using the gravimetric analysis shown earlier\(^8^4\). The calibration set included undersaturated as well as supersaturated samples; the solute concentration in the five different calibration experiments equalled 17.9, 15.9, 13.9, 11.9 and 10.1 g/kg and the temperature values ranged from 35°C to 5.4°C. Baseline corrected spectra were used to develop a partial least-squares regression (PLSR) model that included four latent variables. The root-mean-square error of cross-validation (RMSECV), a measure for the quality of a calibration model, equalled 0.045 g/kg corresponding to 0.3% of the mean of the concentrations as used in the calibration set, which is a rather satisfactory value.

The particle size distributions (PSDs) were measured using a Multisizer 3 from Beckman Coulter based on at least 30,000 particles and were smoothed using a moving average filter. Measurements were carried out using saturated suspensions to which 2 wt.% of NaCl was added to provide the free ions required for this measurement technique.

6.2.4 Experimental procedure

The seeded batch desupersaturation experiments were performed at 20°C in the reactor described in Section 6.2.2. The stirring rate in all
experiments equalled 200 rpm. A supersaturated solution was created by pouring a hot solution into a cold reactor, whose jacket temperature was gradually increased until the temperature of the solution reached the operating temperature of 20°C. During this cooling period of approximately 5 minutes, no significant nucleation occurred, i.e. no sudden increase in the small chord lengths was ever observed in the FBRM signal.

Crystal breakage could be avoided by using a low suspension density and a glass impeller with rounded stirring blade tips operated at low stirring rates. Mild agglomeration was observed as discussed later.

The first ATR-FTIR and Raman spectroscopy measurements were recorded upon addition of the seed crystals, i.e. as soon as the temperature of the solution reached 20°C. Figure 6.3 shows two measured solute concentration profiles using the same initial conditions. The solid concentration is determined from the material balance. It can be seen that the repeatability of the experiments is satisfactory.

6.3 Modeling the spectroscopic data

6.3.1 Estimating the model parameters

In this section, the analysis of time-resolved spectroscopic data, which is the same for ATR-FTIR and for Raman spectroscopy, through a multivariate approach is discussed briefly. A detailed discussion about the notation and multivariate calibration-free analysis of spectroscopic data can be found in Section 3.1 and Section 3.1.2, respectively.

As discussed in the introduction, we assume that the spectra measured in situ scale linearly with the amount of scattering material per unit volume
6.3 Modeling the spectroscopic data

Figure 6.3: The repeatability of the performed experiments indicated by the measured time-resolved solute concentrations obtained through the recorded ATR-FTIR spectra combined with the developed calibration model (filled symbols). The solid concentrations (open symbols) were determined from the mass balance and the experiments were performed at 20°C and 200 rpm.

in the case of Raman spectroscopy and with the solute concentration in the case of ATR-FTIR spectroscopy. Both the IR and the Raman spectra were baseline corrected and the latter were also normalized to reduce variation of the signal due to changes in the process variables such as the particle size of the crystals\textsuperscript{65,70}.

The measured signal intensity at a certain Raman shift or wavenumber $\lambda$ and at a given time $t$, $x(t, \lambda)$, can then be expressed as a linear combination of the signals corresponding to each analyte, i.e.:

$$x(t, \lambda) = \sum_{l=1}^{d} c_l(t)a_l(\lambda) + e(t, \lambda) = \hat{x}(t, \lambda) + e(t, \lambda) \quad (6.1)$$

where $a_l(\lambda)$ represents the intensity at Raman shift or wavenumber, $\lambda$, of the pure-analyte spectrum corresponding to the $l^{th}$ analyte, $c_l(t)$ denotes the concentration of the $l^{th}$ analyte at time $t$, and $e(t, \lambda)$ represents the experimental error, i.e. the noise and the nonidealities of the measure-

By discretizing the spectral and time coordinates, the spectral matrix \( \mathbf{X} \) \((n \times m)\) can then be written in matrix notation as:

\[
\mathbf{X} = \mathbf{CA} + \mathbf{E} = \hat{\mathbf{X}} + \mathbf{E}.
\]

The element \( x_{ij} \) of the matrix \( \mathbf{X} \) represents the \( j^{th} \) measured intensity for the \( i^{th} \) sample and \( \mathbf{C} \) \((n \times d)\) represents the state matrix, i.e. the concentrations of the \( d \) analytes involved, where the \( l^{th} \) column of \( \mathbf{C} \) is the concentration profile in time of the \( l^{th} \) analyte. The \( l^{th} \) row in the matrix \( \mathbf{A} \) \((d \times m)\) denotes the discretized pure-analyte spectrum for analyte \( l \); the matrix \( \mathbf{E} \) \((n \times m)\) is the matrix of experimental errors. It is worth noting that the discretization of the Raman shift or wavenumber coordinate as well as the sampling in time are determined by the settings of the instruments, i.e. the spectrometers.

The key assumption is that the process can be described by a model that predicts how the concentrations of the different analytes evolve in time, i.e. it provides a modeled time-resolved concentration matrix \( \hat{\mathbf{C}}(\mathbf{k}) \), where the vector \( \mathbf{k} \) consists of \( p \) model parameters that are physicochemical or transport properties of the system. The classical approach is to develop a calibration model \( \mathbf{B} \) and to use this calibration to calculate the concentration matrix \( \mathbf{C} = \mathbf{XB} \) from the measured spectral matrix \( \mathbf{X} \). One can then estimate the model parameters \( \mathbf{k} \) by minimizing in some sense the difference between the matrices \( \hat{\mathbf{C}}(\mathbf{k}) \) and \( \mathbf{C} \). This classical approach relies on a calibration procedure, which is often very time-consuming.

One can however also use the time-resolved spectra, i.e. the matrix \( \mathbf{X} \) itself, to estimate directly the model parameters without converting explicitly the spectral matrix \( \mathbf{X} \) into the measured concentrations \( \mathbf{C} \). Formally, this would imply minimizing for instance the sum \( S_r \) of the
squares of the elements of the residual matrix \( \mathbf{R}(k) \) \((n \times m)\):

\[
\mathbf{R}(k) = \mathbf{X} - \hat{\mathbf{C}}(k) \mathbf{A}
\]  

(6.3)

where both the \( p \) elements of the vector \( k \) and the \( d \times m \) elements of the matrix \( \mathbf{A} \) are unknown and should be obtained through some optimization procedure, i.e. a rather challenging task considering that the number of elements of \( \mathbf{A} \) can be very large. However, using the pseudoinverse matrix of \( \hat{\mathbf{C}}(k) \), i.e., \( \hat{\mathbf{C}}^+(k) = (\hat{\mathbf{C}}^T(k)\hat{\mathbf{C}}(k))^{-1}\hat{\mathbf{C}}^T(k) \), the expression can be rewritten as:

\[
\mathbf{R}(k) = \mathbf{X} - \hat{\mathbf{C}}(k)\hat{\mathbf{C}}^+(k)\mathbf{X} = [\mathbf{I} - \hat{\mathbf{C}}(k)\hat{\mathbf{C}}^+(k)]\mathbf{X}.
\]  

(6.4)

By minimizing the sum of the squares of the elements of the residual matrix \( \mathbf{R}(k) \), \( S_r \), one obtains the \( p \) unknown model parameters, i.e. a much easier task than solving eq. (6.3)\textsuperscript{39,41,49,54}.

It is worth noting that for IR spectroscopy, the state matrix \( \hat{\mathbf{C}}(k) \) contains only the solute concentration over time hence its dimensions are \((n \times 1)\). The measured Raman signal is influenced by the solid as well as by the liquid phase, therefore in this case the state matrix \( \hat{\mathbf{C}}(k) \) contains the solid and the solute concentrations over time and its dimensions are \((n \times 2)\). On this basis, the proposed approach enables direct estimation of the solid as well as of the solute concentration when applied to time-resolved Raman spectra\textsuperscript{49}.

### 6.3.2 Parameter estimation techniques

For the estimation of the parameters from the problem defined in eq. (6.4), optimization algorithms such as the Newton-Gauss-Levenberg/Marquardt (NGL/M) and the simplex method approach
were employed using the \textit{lsqnonlin} and \textit{fminsearch} functions in MATLAB, respectively\textsuperscript{35}. The sensitivity of the obtained parameters is indicated by their confidence intervals, i.e. the smaller the confidence interval the smaller the sensitivity of the parameter\textsuperscript{54}. Approximate confidence intervals can be calculated using the sensitivity matrix or Jacobian based on a linearized model in the vicinity of the estimated model parameters. This sensitivity matrix enables to calculate the covariance matrix of the parameter estimates. The standard error $s_k$ of the $k^{th}$ parameter $k_k$ is given by the square root of the $k^{th}$ diagonal element of this covariance matrix. The confidence interval of the $k^{th}$ parameter is given by $k_k \pm t_{\alpha,\nu}s_k$, where $t_{\alpha,\nu}$ is the value of the t-distribution for $\nu$ degrees of freedom, i.e. the number of data points minus the number of parameters, and a confidence level of $\alpha$. Here $\alpha = 0.05$ was used, thus providing a 95\% probability.

### 6.4 Modeling the crystallization process

The mathematical description of the crystallization process is based on the population balance equation (PBE), where proper constitutive equations for the rates of the key mechanisms are plugged in. Such a model can be used to calculate the solid phase concentration, the solute concentration and the particle size distribution of crystals, thereby providing the time-resolved information that forms the columns of the matrix $\hat{C}(k)$.

The PBE for a perfectly mixed batch crystallizer with constant suspension volume and assuming size-independent growth and neither agglomer-
6.4 Modeling the crystallization process

Modification nor breakage reads as follows:

\[ \frac{\partial n}{\partial t} + G \frac{\partial n}{\partial L} = 0 \quad (6.5) \]

where \( L \) is the particle size, \( t \) is the time, \( n \) is the number density of crystals, i.e. its particle size distribution (PSD), and \( G \) denotes the crystal growth rate. Although size-independent crystal growth is not general, it has been shown to be adequate for paracetamol\(^{102}\). The solute material balance, which defines the solute concentration, \( c(t) \), is written as:

\[ \frac{dc}{dt} = -3k_v \rho G \int_0^\infty L^2 n(t,L) dL \quad (6.6) \]

where \( k_v \) and \( \rho \) denote the volume shape factor and the crystal density, respectively. In the case of paracetamol, we have chosen \( k_v = 0.7 \) and \( \rho = 1293 \text{ kg/m}^3 \) based on literature data\(^{102}\). The following initial and boundary conditions apply to the PBE and to the material balance:

\[ n(0,L) = n_0(L) \quad (6.7) \]
\[ n(t,0) = 0 \quad (6.8) \]
\[ c(0) = c_0 \quad (6.9) \]

where \( c_0 \) and \( n_0(L) \) denote the initial solute concentration and the initial particle size distribution, respectively, and it is assumed that no nucleation occurs. The supersaturation, \( S \), required to calculate the crystal growth rate, is defined as \( S = \frac{c}{c^*} \) with \( c^* \) being the solubility. The solubility of paracetamol was measured previously, and at 20°C equals 12.8 g/kg of water\(^{84}\).

The PBE can be solved using a number of approaches; in this work the method of moments is applied\(^{45-47}\). The \( j^{th} \) moment of the PSD of a
population of crystals is defined as

\[ \mu_j(t) = \int_0^\infty L^j n(t, L) dL. \]  \hfill (6.10)

In case no nucleation occurs, the time derivative of the zeroth moment equals zero:

\[ \frac{d\mu_0}{dt} = 0; \] \hfill (6.11)

whereas the equations for all higher moments are:

\[ \frac{d\mu_j}{dt} = jG\mu_{j-1} \quad (j \geq 1). \] \hfill (6.12)

Using the second and third moment, the solute mass balance eq. (6.6) can be recast as:

\[ \frac{dc}{dt} = -3k_v \rho G\mu_2 = -k_v \rho \frac{d\mu_3}{dt}. \] \hfill (6.13)

The solid concentration of crystals, \( m(t) \), can be calculated using the third moment \( m(t) = k_v \rho \mu_3(t) \), and by combining this with eq. (6.13) one obtains

\[ c(t) = c_0 + m_0 - m(t), \] \hfill (6.14)

where \( m_0 \) denotes the mass of seed crystals, i.e. \( m(0) \).

Eqs. (6.11) and (6.12), for \( j = 1, 2, 3 \), form a system of four coupled ordinary differential equations combined with the mass balance eq. (6.14), which can be solved once the initial values of the four moments of the PSD and of the solute concentration are fixed.

The particle size distribution at any time \( t \) can be reconstructed using the following expression, which can be obtained using the recently proposed Laplace transform technique\(^{45}\) as well as through the method of

\[ \text{128} \]
characteristics:  

\[ n(t, L) = n_0 \left( L - \int_0^t G(\tau) d\tau \right) \]  

(6.15)

where the integral appearing in the r.h.s. can be calculated once the evolution of the solute concentration over time, \( c(t) \), is known.

Several approaches exist to express the growth rate \( G \) as a function of the supersaturation \( S \) as described in detail elsewhere. In this work, the objective was not to provide a first-principle and temperature dependent growth rate expression based on a specific crystal growth mechanism, but to demonstrate that crystal growth rate parameters can be obtained through fitting time-resolved spectroscopic data and thereby avoiding lengthy calibration procedures. For this reason, a relatively simple, empirical power-law expression has been employed to describe the crystal growth rate as function of the supersaturation:

\[ G = k_1 (S - 1)^{k_2}. \]  

(6.16)

### 6.5 Results and discussion

Three different runs were performed all with a seed mass and a solvent mass of 0.4 g and 200 g, respectively. In run 1, 2 and 3 the initial supersaturation equalled 1.18, 1.23, 1.28, respectively. First, the classical approach to obtain growth rate parameters, i.e. fitting the desupersaturation profiles measured using ATR-FTIR spectroscopy combined with a calibration, will be presented. Then, the growth rate parameters are estimated through fitting the measured time-resolved IR spectra and finally through fitting the measured time-resolved Raman spectra. The
parameters resulting from these three approaches will be compared and discussed in the context of other studies.

### 6.5.1 Fitting measured desupersaturation profiles

Figure 6.4 shows for the three different runs the measured desupersaturation profiles (symbols), obtained through the ATR-FTIR spectra and the developed calibration model. The lines represent the modeled desupersaturation profiles resulting from the fitting procedure as discussed in Section 6.3.1. As it can be seen, the agreement between the measured and the modeled desupersaturation profiles is satisfactory. The estimated growth rate parameters are reported in the first row of Table 6.1.

![Figure 6.4: The measured (symbols) and modeled (lines) desupersaturation profiles for the three runs. In all experiments, the temperature and stirring rate equalled 20°C and 200 rpm, respectively.](image)

It is worth noting that the initial supersaturation is limited by the necessity to avoid that crystals stick to the ATR probe window, as reported in the case of other ATR-FTIR studies\textsuperscript{52,75,84}. For supersaturation levels higher than those reported in this work, crystals were found to attach to
the ATR probe window, thereby hindering the quantitative application of in situ ATR-FTIR spectroscopy. Though in principle there is no lower bound for the initial supersaturation, a minimum change in solid mass is required to enable the analysis of the in situ Raman spectra as discussed below.

The final particle size distributions of all experiments were measured and compared to the simulation results using these estimated crystal growth rate kinetics. Figure 6.5 illustrates such comparison for run 3; the measured PSDs of the seed crystals and of the product crystals (symbols) are shown together with the calculated final PSD. It should be noted that the measured particle size distributions (symbols) result from Multisizer measurements and the simulated one (line) is obtained using the mathematical model and the estimated parameters. It can readily be observed that the measured final PSD is slightly broader and shifted towards larger particle sizes as compared to the calculated PSD. This indicates, despite the very low suspension density of 0.2 wt.%, mild agglomeration of crystals, as also observed in microscopic images of the product crystals. It is worth noting that the samples for particle size characterization measurements were drawn directly from the reactor, thereby omitting filtration and drying steps that could affect the particle size distribution of crystals.

It was shown previously\textsuperscript{52,86} that mild agglomeration does not affect the desupersaturation profile significantly. On this basis, and considering that the proposed method uses only information about solute and solid concentrations, the assumptions of the process model, i.e. nucleation, agglomeration and breakage are not taken into account, are justified.

Figure 6.5: The measured (filled symbols) and the simulated (line) particle size distributions at the end of run 3. The particle size distribution of the seed crystals (open symbols) is shown for comparison. The measured particle size distributions (symbols) result from Multisizer measurements and the simulated one (line) is obtained using the mathematical process model and the parameters reported in the first row of Table 6.1.

6.5.2 Fitting the measured time-resolved spectra

As discussed in Section 6.3.1, the kinetic parameters can also be obtained through fitting the measured time-resolved spectra assuming the spectra scale linearly with the concentrations. Parts a, b, c and d of Figure 6.6 illustrate this approach for the measured time-resolved IR spectra, whereas the parts e, f, g and h serve the same purpose for the measured time-resolved Raman spectra. In both cases, run 3 is analyzed. The corresponding estimated crystal growth rate parameters when analyzing all runs are reported in the second and third row of Table 6.1, respectively. It should be emphasized that in the case of IR spectroscopy, the state matrix \( \hat{\mathbf{C}}(k) \) contains only the solute concentration over time hence its dimensions are \((n \times 1)\). In the case of Raman spectroscopy the signal is influenced by the liquid as well as the solid phase hence the state matrix \( \hat{\mathbf{C}}(k) \) contains the solute and the solid concentrations over
time and its dimensions are \((n \times 2)\). Due to eq. (6.14) these quantities are not independent and the solute concentration and the concentration of solid are directly connected. On this basis, the difference between the application of this approach to in situ ATR-FTIR and to in situ Raman spectroscopy is not expected to have a major effect on the accuracy of the estimated parameters.

Figures 6.6(a) and 6.6(e) show the measured time-resolved IR and Raman spectra, respectively. As it can be seen, the intensities of the IR spectra decrease after the seed crystals are added to the supersaturated solution and the supersaturation is consumed by crystal growth, as expected. In the case of the time-resolved Raman spectra, a slight shift towards lower Raman shifts can be observed. This shift in the Raman shift coordinate is due to increasing and decreasing peaks as often encountered in the application of in situ Raman spectroscopy, e.g. during polymorph transformations\(^{20,84}\). In this case, the shift results from a decreasing peak due to the decreasing solute concentration and an increasing signal resulting from an increasing suspension density.

Figures 6.6(b) and 6.6(f) display the measured and the modeled intensities at different wavenumbers and Raman shifts, respectively. The agreement between the measured and the modeled intensities is in both cases satisfactory. The Raman intensities are relatively scattered due to the low suspension density. If the suspension density were higher, the supersaturation would be consumed in a matter of a few measurements and the presented analysis would not be possible.

The modeled concentrations that result from fitting the time-resolved spectra in the case of IR and Raman spectroscopy can be found in Figures 6.6(c) and 6.6(g), respectively. It is worth emphasizing that this approach establishes a direct link between the measured spectra, i.e. the matrix \(X\), and the model parameters, i.e. the vector \(k\), without
Figure 6.6: Fitting spectroscopic data in the case of ATR-FTIR spectroscopy (parts a, b, c and d) and Raman spectroscopy (parts e, f, g and h). In both cases, run 3 is analyzed. The time-resolved IR and Raman spectra are shown in the parts a and e, respectively. Parts b and f demonstrate the agreement between the measured (symbols) and the modeled (lines) intensities for IR and Raman spectroscopy, respectively. The estimated concentrations are shown in parts c and g. The resulting pure-analyte spectra (continuous lines) are compared to the measured ones (dashed lines) in the parts d and h for IR and Raman spectroscopy, respectively.
6.5 Results and discussion

an explicit estimation of the state variables, i.e. $C$. The latter can be known only by letting the model calculate them as it is done to draw the plots in Figures 6.6(c) and 6.6(g).

Finally, Figures 6.6(d) and 6.6(h) show the calculated pure-analyte spectra (continuous lines) and the measured pure-analyte spectra (dashed lines) in the case of IR and Raman spectroscopy, respectively. It should be noted that these pure-analyte spectra represent a concentration of 1 g/kg. The measured spectra result from independent measurements and the modeled ones are obtained directly from the fitting of the time-resolved spectra. As it can be seen, the agreement between the modeled and the measured spectra is excellent.

6.5.3 Comparison of different modeling approaches

With reference to run 3, Figure 6.7 shows the comparison of the desupersaturation profiles calculated using the parameters estimated through the three different approaches described above and reported in Table 6.1. The symbols represent the measured concentrations that are obtained through the developed calibration model for the IR spectra as discussed in Section 6.2.3. The modeled solute concentration profiles resulting from fitting the desupersaturation profiles and from fitting the time-resolved IR spectra are entirely overlapping. This demonstrates the applicability of fitting measured time-resolved IR spectra and that essentially the same process model can be obtained without time-consuming calibration procedures. Small differences can be observed between both approaches resulting from the time-resolved IR spectra and the time-resolved Raman spectra. However, the agreement between the modeled concentrations resulting from fitting the time-resolved Raman spectra and the measured concentrations is satisfactory.
Figure 6.7: The estimated concentration profiles resulting from the three different modeling approaches (lines) and the measured concentrations (symbols) obtained from the calibration model applied to the IR spectra for run 3.

Table 6.1 summarizes the estimated parameters obtained by fitting the experimental data resulting from the three runs as described above. The parameters from fitting the desupersaturation profiles and from fitting the time-resolved IR spectra are very similar as could be expected from Figure 6.7. The parameters estimated through fitting the time-resolved Raman spectra show a discrepancy compared to the other two sets of parameters. However, the agreement between the modeled and the measured concentrations is still satisfactory as shown in Figure 6.7. The discrepancy between the parameters could be due to the correlation between $k_1$ and $k_2$. Additionally, Figures 6.6(b) and 6.6(f) show that the scattering of the measured infrared spectra is different compared to the scattering of the measured Raman spectra which might also shift the global minimum and therefore yield slightly different parameters.

Figure 6.8 plots the growth rate as a function of the supersaturation for the three different modeling approaches as continuous lines. As expected, the growth rate estimated from fitting the desupersaturation profiles and from fitting the time-resolved infrared spectra are almost identical. The
6.5 Results and discussion

<table>
<thead>
<tr>
<th></th>
<th>$k_1 \cdot 10^6 \text{ [m s}^{-1}\text{]}$</th>
<th>$k_2 \text{ [-]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration-based, supersaturations</td>
<td>0.68 ± 0.04</td>
<td>1.29 ± 0.03</td>
</tr>
<tr>
<td>Calibration-free, IR spectra</td>
<td>0.67 ± 0.03</td>
<td>1.26 ± 0.02</td>
</tr>
<tr>
<td>Calibration-free, Raman spectra</td>
<td>0.39 ± 0.03</td>
<td>1.04 ± 0.03</td>
</tr>
</tbody>
</table>

Table 6.1: The estimated crystal growth rate parameters resulting from the three different modeling approaches applied to the three experiments described above. All optimizations were performed using the simplex algorithm.

growth rate estimated from the time-resolved Raman spectra differs, especially for higher supersaturation values, from the growth rate estimated through the time-resolved IR spectra and from the one estimated through the measured desupersaturation profiles as expected from the parameter values given in Table 6.1. This discrepancy could be caused by the fact that only few measurements are available for the parameter estimation for supersaturation values larger than 1.15 as it can be seen in Figure 6.4. The key result is however that very similar process models can be developed through fitting the time-resolved IR spectra and through fitting the time-resolved Raman spectra, thereby avoiding extensive calibration procedures for both in situ spectroscopic techniques.

6.5.4 Comparison with literature data

Granberg and Rasmuson\textsuperscript{102} estimated the growth rate of paracetamol crystals in different solvents at 16\textdegree C through fitting concentration data obtained by gravimetric analysis. The estimated growth rate in water is shown in Figure 6.8 as dashed line. The growth rates estimated in this work are in the same range, however, differences are evident. Numerous reasons for this discrepancy can be conjectured. The first is that in the two studies the range of the initial supersaturation levels, and thereby

Figure 6.8: The estimated growth rates as a function of the supersaturation (continuous lines) at 20°C compared to the estimated growth rate at 16°C taken from the literature\textsuperscript{102} (dashed line). The growth rates are given in the range of supersaturation values that were used for the parameter estimation. It should be noted that the overall growth rate of a volume equivalent sphere is plotted for all cases.

the supersaturation values employed for the parameter estimation, was different, i.e. in the previous work the initial supersaturation equalled 1.18 whereas in this work a maximum initial supersaturation of 1.28 was applied. The second reason is a difference in the temperature at which the experiments were performed, i.e. $T = 16^\circ$C for the dashed line whereas our results where obtained at 20°C. For supersaturation values lower than 1.12 both sets of growth rates are indeed consistent, i.e. a lower temperature results in a lower growth rate. However for higher supersaturation values, our results predict a lower growth rate. A possible explanation for this difference is the fact that in the work by Granberg and Rasmuson far less measurement points are available due to the experimental approach, i.e. gravimetric analysis of samples drawn during only one experiment. Inspection of Figure 6.4 demonstrates that our results are based on three experiments resulting in hundreds of measured concentrations due to the use of in situ spectroscopic techniques.
Finally, the seed crystals in Granberg and Rasmuson were allowed to reshape or to ripen before being used in the batch experiments. This might have smoothed the crystal faces and dissolved possible impurities adsorbed on them, thereby changing the crystal growth rate slightly.

It should be emphasized that the objective of this work was not to provide the most accurate process model for the crystal growth of paracetamol in water at $20^\circ$C, but to demonstrate that very similar process models can be obtained by fitting the measured desupersaturation profiles, by fitting the measured time-resolved IR spectra and by fitting the measured time-resolved Raman spectra.

### 6.6 Conclusions

Using seeded batch desupersaturation experiments and a population balance model, it was demonstrated that by fitting the measured time-resolved ATR-FTIR spectra directly and by fitting the desupersaturation profiles obtained from these spectra combined with a previously developed calibration model, essentially the same crystal growth rate parameters for paracetamol in water can be estimated. Furthermore, the measured time-resolved Raman spectra were also fitted directly using the same population balance model and very similar crystal growth rate parameters could be determined. Knowing that crystals attach to ATR probe windows more frequently than they do to in situ Raman probes, the latter achievement can be of major importance in the characterization of kinetic phenomena in crystallization processes. More in general, these results show that in the case a descriptive process model is available both in situ spectroscopic techniques can be applied in a calibration-free manner, thereby avoiding time-consuming and costly calibrations.
Chapter 7

The direct inverse method: a new method to estimate isotherm parameters

A novel method to estimate adsorption isotherm parameters is presented and its applicability is studied through synthetic as well as experimental data. This approach assumes a linear dependency of the UV absorption intensity on the solute concentration in the fluid phase, at least in certain ranges of the UV spectra. It was demonstrated that by fitting

the absorption profiles, i.e. the new direct inverse method, and by fitting the concentration profiles, i.e. the classical inverse method, very similar adsorption isotherm parameters can be obtained. The findings presented in this work have as important implication the elimination of the requirement of converting a measured absorption intensity into a concentration value, i.e. the elimination of the calibration of the UV signal. The second implication is the elimination of the requirement of a fraction analysis in the case of overlapping components.

### 7.1 Introduction

Liquid chromatography is frequently applied as a chemical separation process to isolate one or more components from a mixture. Its separation mechanism is a consequence of the difference between the retention characteristics of the individual components in the mixture. Elucidation of the adsorption isotherms and estimation of their parameters is a crucial step toward designing an optimal preparative chromatographic process.

Several methods exist to characterize adsorption isotherms through chromatography, among them frontal analysis, the elution by characteristic point and the perturbation method\(^{107}\). However, these methods usually estimate single component isotherms, require a significant amount of experimental effort and material. One method that allows estimation of single and multi component competitive isotherm parameters with little experimental effort, is the inverse method or classical inverse method (CIM) as it is referred to throughout this work. In this method, the adsorption isotherm parameters are obtained by fitting simulated chromatograms to experimental ones. This method has successfully been
applied to determine adsorption isotherm parameters for a manifold of components using various adsorption isotherms\textsuperscript{107–112}. However, there are several drawbacks of this classical inverse method. In the case components are separated, the signal of the detector, e.g. an ultraviolet (UV) spectrometer, requires calibration, which is a time-consuming and costly exercise. In the case where components are overlapping, fraction collection and subsequent analysis of these fractions, which itself also requires calibration of the UV signal, is the most common way to obtain the elution profiles of the individual components in order to facilitate the application of the classical inverse method.

In this work, a novel method to estimate adsorption isotherm parameters, the direct inverse method (DIM), is presented. The proposed approach is a modification of the classical inverse method and uses directly the elution profiles at multiple wavelengths provided by the UV spectrometer or detector without any calibration efforts. Moreover, the operating principles of the DIM are equally applicable in the case of strongly overlapping components and therefore eliminate the necessity of fraction analysis which is also very time-consuming. It is worth noting that both the classical and the direct inverse method require the selection of a specific isotherm, i.e. a specific functional form to describe adsorption. Such a choice can be made based on a series of overloaded pulse injections starting with the simplest isotherms and using more complicated ones when the former do not work well enough\textsuperscript{107}.

The direct inverse method has similarities with a recent study that estimates the adsorption isotherm parameters using the sum of the concentrations of two enantiomers\textsuperscript{109}. The method proposed in that work however, works only in the case of enantiomers that have identical UV spectra and still requires calibration. In another work, the sum of the absorptions is used to estimate the concentrations of two components
having different UV spectra through the solution of an optimization problem. Also in that study, a calibration for both components was required. The novel direct inverse method works in both the cases, i.e. for enantiomers and for components that have different UV spectra, and does not require any calibration efforts.

The main assumption and requirement of the proposed direct inverse method is that for all the involved components, the measured UV absorption scales linearly with the solute concentration in the fluid phase. It is known that this is certainly not the case for all the wavelengths, however over small ranges the absorption increases linearly with the solute concentration in the fluid phase as it is shown in Figures 7.1(a) and 7.1(b) for the two components used in this work, i.e. phenetole (PHT) and 4-tert-butylphenol (TBP), respectively.

Figure 7.1: UV intensity as a function of the solute concentration in the fluid phase in the case of phenetole (component 1) (a) and of 4-tert-butylphenol (component 2) (b), respectively. All measurements were performed at 22°C by injecting 5 mL of the solution without the installation of a column and the values shown are the values of the resulting plateaus.

First, synthetic data is used to demonstrate the effectiveness of this novel method. Subsequently, experimental data are used to show that very
similar adsorption isotherm parameters can be obtained when fitting directly the time-resolved UV spectra and when fitting the time-resolved concentrations of the individual components. These elution profiles are obtained either through calibration of the UV signal or through fraction analysis in the case of overlapping components.

7.2 Experimental

7.2.1 System

All data was acquired using an Agilent (Palo Alto, CA, USA) LC System 1100 Series equipped with a multisolvent delivery system, an autosampler, a column thermostat, a diode array UV detector and an automated data acquisition system. The extra-column volume equalled 0.078 mL and has been accounted for in all data reported. In all the experiments, the temperature in the column thermostat was kept constant at a value of 22°C. The flow rate in all the experiments equalled 1 mL/min.

7.2.2 Chemicals

Phenetole or ethoxybenzene and 4-tert-butylphenol were purchased from Sigma-Aldrich (Buchs, Switzerland) and used without further purification. Uracil or 2,4-dihydroxypyrimidine, also purchased from Sigma-Aldrich, was used as a tracer to determine the extra-column volume of the system and the overall bed void fraction of the column. A methanol-water mixture (65:35 v/v) was used as the mobile phase in all the experiments. Deionized water was obtained from a Milli-Q Advantage A10 water purification system from Millipore (Zug, Switzerland) and HPLC grade methanol was purchased from Sigma-Aldrich.
7.3 Modeling the spectroscopic data

7.2.3 Column

The chromatographic column used was a C18 Zorbax-StableBond300 from Agilent (150 $\times$ 4.6 mm). The column overall bed void fraction was estimated to be 0.62 using uracil as a tracer compound. During the experiments, fractions were collected using a FC203B fraction collector from Gilson (Mettmenstetten, Switzerland).

7.3 Modeling the spectroscopic data

7.3.1 Notation

In this section, the proposed method to estimate adsorption isotherm parameters, i.e. the direct inverse method (DIM), will be discussed in detail. The proposed method is similar to a novel approach to estimate kinetic parameters from Raman or infrared spectra applied to a crystallization process\(^{49}\), and hence a very similar notation will be used.

We assume that the measured UV spectra, which were used without any preprocessing, scale linearly with the concentration of all $d$ components. The measured signal intensity at a certain wavelength $\lambda$ and at a given time $t$, $x(t, \lambda)$, can then be expressed as a linear combination of the signals corresponding to each component, i.e.:

$$x(t, \lambda) = \sum_{l=1}^{d} c_l(t) a_l(\lambda) + e(t, \lambda) = \hat{x}(t, \lambda) + e(t, \lambda) \quad (7.1)$$

where $a_l(\lambda)$ represents the intensity at wavelength, $\lambda$, of the pure-component spectrum corresponding to the $l^{th}$ component, $c_l(t)$ denotes the concentration of the $l^{th}$ component at time $t$, and $e(t, \lambda)$ represents
7. The direct inverse method: a new method to estimate isotherm parameters

the experimental error, i.e. the noise and the nonidealities of the measurement. By discretizing the spectral and the time coordinates, the spectral matrix $X$ ($n \times m$) can then be written as:

$$X = CA + E = \hat{X} + E. \quad (7.2)$$

The element $x_{ij}$ of the matrix $X$ represents the $j^{th}$ measured intensity for the $i^{th}$ sample and $C$ ($n \times d$) represents the state matrix, i.e. the concentrations of the $d$ components involved, where the $l^{th}$ column of $C$ is the concentration profile in time of the $l^{th}$ component. The $l^{th}$ row in the matrix $A$ ($d \times m$) denotes the discretized pure-component spectrum for component $l$; the matrix $E$ ($n \times m$) is the matrix of experimental errors. It is worth noting that the discretization of the wavelength coordinate as well as the sampling in time are determined by the settings of the instrument, i.e. the spectrometer.

7.3.2 Classical inverse method

In the classical inverse method (CIM), the adsorption isotherm parameters are obtained by fitting simulated chromatograms, i.e. the time-evolution of the concentrations of the individual components, to the experimental ones. Therefore, the CIM requires a calibration of the UV signal that converts the time-evolution of the intensity at one wavelength into the time-evolution of the concentration of a certain component:

$$c_l = x_j b_{lj} \quad (7.3)$$

where $c_l$ denotes the time-evolution of the concentration of component $l$, $x_j$ is the $j^{th}$ column of the spectral matrix $X$, and $b_{lj}$ represents the calibration factor for component $l$ at the wavelength $j$. Separate sets
of injections are required to determine $b_{ij}$ for each component. The CIM then employs these calibration factors to estimate $c_l$ for all the components and the measured state-matrix $C$ can easily be obtained by placing the column vectors $c_l$ next to each other.

The next step is the availability of a model that can predict the time-evolution of the concentrations of all components, i.e. that can provide a modeled time-resolved concentration matrix $\hat{C}(k)$, where $k$ consists of $p$ model parameters that are physicochemical or transport properties of the system, e.g. adsorption isotherm parameters or dispersion coefficients.

The classical inverse method estimates the model parameters $k$ by minimizing in some sense the difference between the matrices $\hat{C}(k)$ and $C$. It is clear that this classical approach relies on a calibration procedure, which is often very time-consuming\textsuperscript{107}.

### 7.3.3 Direct inverse method

One can however also use the time-resolved UV elution spectra, i.e. the matrix $X$ itself, to estimate directly the model parameters without converting explicitly the spectral matrix $X$ into the measured concentrations $C$. Formally, this would imply minimizing for instance the sum $S_r$ of the squares of the elements of the residual matrix $R(k)$ ($n \times m$):

$$R(k) = X - \hat{C}(k)A$$

where both the $p$ elements of the vector $k$ and the $d \times m$ elements of the matrix $A$ are unknown and should be obtained through some optimization procedure, i.e. a rather challenging task considering that the number of elements of $A$ can be large. However, using the pseudoinverse matrix of $\hat{C}(k)$, i.e., $\hat{C}^+(k) = (\hat{C}^T(k)\hat{C}(k))^{-1}\hat{C}^T(k)$, eq. (7.4) can be
rewritten as:

\[ \mathbf{R}(k) = \mathbf{X} - \hat{\mathbf{C}}(k)\hat{\mathbf{C}}^+(k)\mathbf{X} = [\mathbf{I} - \hat{\mathbf{C}}(k)\hat{\mathbf{C}}^+(k)]\mathbf{X}. \]  

(7.5)

By minimizing the sum of the squares of the elements of the residual matrix \( \mathbf{R}(k) \) one obtains the \( p \) unknown model parameters, i.e. a much easier task than solving eq. (7.4)\textsuperscript{39,49,54}.

### 7.3.4 Parameter estimation techniques

For the estimation of the parameters from the problem defined in eq. (7.5), optimization algorithms such as the Newton-Gauss-Levenberg/Marquardt (NGL/M) and the simplex method approach were employed using the \texttt{lsqnonlin} and \texttt{fminsearch} functions in MATLAB, respectively\textsuperscript{35}. The sensitivity of the obtained parameters is indicated by their confidence intervals, i.e. the smaller the confidence interval the smaller the sensitivity of the parameter\textsuperscript{54}. Approximate confidence intervals can be calculated using the sensitivity matrix or Jacobian based on a linearized model in the vicinity of the estimated model parameters. This sensitivity matrix enables to calculate the covariance matrix of the parameter estimates. The standard error \( s_i \) of the \( i^{th} \) parameter \( k_i \) is given by the square root of the \( i^{th} \) diagonal element of this covariance matrix. The confidence interval of the \( i^{th} \) parameter is given by \( k_i \pm t_{\alpha,\nu}s_i \), where \( t_{\alpha,\nu} \) is the value of the t-distribution for \( \nu \) degrees of freedom, i.e. the number of data points minus the number of parameters, and a confidence level of \( \alpha \). Here \( \alpha = 0.05 \) was used, thus providing a 95% probability.
7.4 Chromatographic model

The equilibrium-dispersive model of chromatography was used to describe the evolution of the concentration of two components over time and space, hence the mass balance for component $i$ can be written as:

$$\epsilon^* \frac{\partial c_i}{\partial t} + (1 - \epsilon^*) \frac{\partial q_i}{\partial t} + u \frac{\partial c_i}{\partial z} = \epsilon^* D_{ap,i} \frac{\partial^2 c_i}{\partial z^2} \quad (i = 1, 2) \quad (7.6)$$

The axial dispersion and mass-transfer resistance are lumped together in an apparent dispersion coefficient term, proportional to $D_{ap,i}$. In eq. (7.6), $c_i$ and $q_i$ are the fluid phase and the equilibrium adsorbed phase concentration, respectively. The phase equilibrium between the fluid and the adsorbed phase is characterized by the adsorption isotherm $q_i = f_i(c_1, c_2)$. The overall bed void fraction and the superficial velocity of the fluid are represented by $\epsilon^*$ and $u$, respectively. The Danckwerts boundary conditions were used to complement eq. (7.6).

Assuming that the apparent dispersion coefficient is the same for all components, i.e. $D_{ap,i} = D_{ap}$, eq. (7.6) can be solved numerically using a finite difference scheme where the axial dispersion is replaced by numerical dispersion. This can be achieved through discretizing the first-order space derivative using backward differences:

$$\frac{c_i(z) - c_i(z - \Delta z)}{\Delta z} = \left. \frac{\partial c_i}{\partial z} \right|_z - \left. \frac{\partial^2 c_i}{\partial z^2} \right|_z \frac{\Delta z}{2} + O(\Delta z^2) \quad (7.7)$$

where the numerical dispersion is proportional to $\Delta z$. By choosing the discretization of the space coordinate such that

$$\Delta z = \frac{2\epsilon^* D_{ap}}{u}, \quad (7.8)$$
the numerical dispersion introduced by neglecting the second term on
the right-hand side of eq. (7.7) will cancel the axial dispersion, i.e. the
right-hand side of eq. (7.6). Eq. (7.8) can easily be fulfilled by choosing
the number of grid points properly, i.e. \( N_G = L/\Delta z \) where \( L \) denotes
the length of the column.

In this work, both components were assumed to be subject to a compet-
titive generalized Langmuir isotherm:

\[
q_i = \frac{H_i c_i}{1 + K_1 c_1 + K_2 c_2},
\]

(7.9)

where \( K_1 \) and \( K_2 \) can be either positive or negative\(^\text{116}\).

7.5 Results and discussion

In this section, the feasibility of the proposed direct inverse method will
be studied thoroughly using synthetic (first part of this section) as well
as experimental data (second part of this section).

7.5.1 Simulation results

Let us consider two components subject to a binary adsorption isotherm
as given by eq. (7.9), where the corresponding isotherm parameters
equal \( H_1 = 10, H_2 = 12, K_1 = 0.1 \) and \( K_2 = 0.1 \). Two chromatographic
separations were simulated differing in the feed concentrations, i.e. \( \mathbf{c}_f \)
\( = [8 \ 10] \ \text{g/l} \) and \( \mathbf{c}_f \ = [20 \ 20] \ \text{g/l} \), where the first and second element of
\( \mathbf{c}_f \) represents the feed concentration of component one and two, respec-
tively. The column characteristics were assumed to be as described in
Section 7.2. The equilibrium-dispersive model was used and the apparent axial dispersion coefficient $D_{ap}$ was assumed to be equal to $5 \times 10^{-6}$ m$^2$ s$^{-1}$ for both components.

To obtain the time-resolved UV elution spectra, i.e. to obtain the spectral matrix $X$, pure-component UV spectra have to be used. First, single Gaussian peaks were assumed as pure-component spectra for both components. Once the concentration profiles were calculated, the spectral matrix could easily be calculated using eq. (7.2) and white noise with a standard deviation of 3 mAu was added in order to generate realistic UV elution spectra.

The time-evolution of the concentrations, the pure-component spectra, the noise and the resulting simulated UV are shown in Figure 7.2.

As discussed in Section 7.3.4, standard optimization algorithms can be used to solve the optimization problem associated to eq. (7.5), i.e. to recover the parameter values initially selected, using UV elution spectra as shown in Figure 7.2(d) and the process model to calculate the concentration matrix $\hat{C}(k)$. Both the Newton-Gauss-Levenberg/Marquardt (NGL/M) and the simplex method resulted in the correct parameters (exactly correct to two significant figures) in case of low as well as high feed concentrations. It should be emphasized that the concentration profiles shown in Figure 7.2(a) are entirely overlapping and that a fraction collection and subsequent fraction analysis would have been required in order to apply the classical inverse method in this case.

It is well-known however, that pure-component UV spectra do not resemble Gaussian peaks and the range of wavelengths where the intensity scales linearly with the concentration is usually found in the tails of the UV signals. Hence the same procedure was carried out using the pure-component spectra shown in Figure 7.3(a). To demonstrate that
7. The direct inverse method: a new method to estimate isotherm parameters

Figure 7.2: The time-evolution of the concentrations (a), the pure-component spectra (b), the noise (c) and the resulting simulated UV elution spectra (d) used in the simulation studies in the case of Gaussian pure-component spectra.
the two pure-component spectra are indeed different, the ratio of both has also been plotted. The resulting simulated UV elution spectra in the case of the low feed concentrations are shown in Figure 7.3(b). It is worth noting that the time-evolution of the concentrations and the noise were the same as in the case of Gaussian peaks. Also in this case, the Newton-Gauss-Levenberg/Marquardt (NGL/M) and the simplex method resulted in the correct parameters (exactly correct to two significant figures) for both low and high feed concentrations.

![Figure 7.3: The pure-component spectra (a) and the resulting simulated UV elution spectra (b) used in the simulation studies in the case of more realistic pure-component spectra. To show that the pure-component spectra are indeed different, the ratio of both has also been plotted (dashed line).](image)

### 7.5.2 Experimental results

Six different experiments were performed using different feed concentrations and injection volumes; the corresponding experimental conditions are reported in Table 7.1. The elution profiles of runs 1 to 3 and of runs 4 to 6 are shown in Figures 7.4(a) and 7.4(b), respectively. As it
can readily be seen, runs 1 and 4 exhibit baseline separation, whereas runs 2 and 5 exhibit moderate overlapping and runs 3 and 6 complete overlapping of the two components.

<table>
<thead>
<tr>
<th>run</th>
<th>Feed concentration [g/l]</th>
<th>Injection volume [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[10 15]</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>[10 15]</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>[10 15]</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>[6 9]</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>[6 9]</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>[6 9]</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 7.1: Experimental conditions of the different runs. All experiments were performed at 22°C. The first element of the feed concentration \( c_f \) represents the feed concentration of phenetole (component 1).

Figure 7.4: The absorption profiles for runs 1 to 3 (a) and for runs 4 to 6 (b).

In this section, all runs are analyzed both through the classical inverse method using the concentration profiles, i.e. obtained either from the elution profiles through a calibration or from the outcome of a fraction analysis, and through the direct inverse method using the absorption profiles only. In the first part, three different sets of runs are considered.
independently, i.e. runs 1 and 4, runs 2 and 5 and runs 3 and 6. In the second part, all runs will be used together to estimate the adsorption isotherm parameters using the classical and the direct inverse method.

It is worth noting that while on the one hand $K_2$ is positive, indicating that 4-tert-butylphenol (component 2) follows a Langmuir isotherm, on the other hand $K_1$ was found to be negative, meaning that phenetole (component 1) is subject to an anti-Langmuir isotherm. As a consequence, this specific binary system is subject to the mixed generalized Langmuir isotherm $M_2$ as introduced earlier.\textsuperscript{116}

Figures 7.5(a) and 7.5(c) show the experimental (symbols) and simulated (lines) concentration profiles in the case of runs 1 and 4, respectively. The estimated adsorption isotherm parameters and dispersion coefficients, i.e. the results of the classical inverse method, are given in the left column of the first part of Table 7.2. Figures 7.5(b) and 7.5(d) display the experimental (symbols) and simulated (lines) absorption profiles for three different wavelengths in the case of runs 1 and 4, respectively. The estimated adsorption isotherm parameters and dispersion coefficients, i.e. the results of the direct inverse method, are given in the right column of the first part of Table 7.2. As it can be seen, the differences between the parameters estimated through the classical and the direct inverse method are small, which demonstrates the potential of the new calibration-free method.

The same sequence of figures is shown in Figure 7.6 and in Figure 7.7 in the case of runs 2 and 5 and in the case of runs 3 and 6, respectively. An important difference with respect to runs 1 and 4 is that the concentration profiles, required to apply the classical inverse method, can not be obtained from the absorption profiles and the calibration because the peaks are overlapping, hence the concentration profiles were obtained using fraction analysis, i.e. a tedious and time-consuming experimental
7. The direct inverse method: a new method to estimate isotherm parameters

procedure. The estimated adsorption isotherm parameters and dispersion coefficients are reported in the second and third part of Table 7.2. As it can be seen, even when the compounds are entirely overlapping, very similar parameter values can be obtained using the direct inverse method, i.e. with neither calibration nor fraction analysis.

Figure 7.5: The results of the classical and the direct inverse method in the case of run 1 (a and c) and run 4 (c and d). The intensities at wavenumbers 292 (highest intensity), 295 and 297 (lowest intensity) nm are plotted.
Figure 7.6: The results of the classical and the direct inverse method in the case of run 2 (a and c) and run 5 (c and d). The concentration values in the parts a and c were obtained using a fraction analysis. The intensities at wavenumbers 292 (highest intensity), 295 and 297 (lowest intensity) nm are plotted.
7. The direct inverse method: a new method to estimate isotherm parameters

Figure 7.7: The results of the classical and the direct inverse method in the case of run 3 (a and c) and run 6 (c and d). The concentration values in the parts a and c were obtained using a fraction analysis. The intensities at wavenumbers 293 (highest intensity), 296 and 298 (lowest intensity) nm are plotted.
Table 7.2: Results of the classical and the direct inverse method using three different sets of experimental data. All optimizations were performed using the simplex algorithm. The values for the dispersion coefficients are given in $10^{-5}$ m$^2$ s$^{-1}$. For reasons of clarity, the subscript $ap$ has been omitted for the dispersion coefficients.
7. The direct inverse method: a new method to estimate isotherm parameters

Of course all experiments can be used together to provide a final estimate for the adsorption isotherm parameters using the classical and direct inverse method. The parameter values of this analysis are given in Table 7.3 and the resulting adsorption isotherms are displayed in Figure 7.8. For reasons of clarity, the estimates for the apparent dispersion coefficients are not given as they were very similar to those given in Table 7.2. As it can be seen, both the isotherms estimated through the classical (continuous lines) and the direct (dashed lines) inverse method, are very similar and only small differences can be observed for higher solute concentrations.

![Figure 7.8: Estimated adsorption isotherms for both components using the classical (continuous lines) and the direct (dashed lines) inverse method.](image)

It is worth noting, that there is always a small discrepancy between the estimated parameter values using the classical and the direct inverse method. However, in the case of the dispersion coefficients, the discrepancy between the two approaches is relatively large, especially in the cases where fraction analysis was required to determine the concentration profiles. In the case of a fraction analysis, additional tubing was
7.5 Results and discussion

<table>
<thead>
<tr>
<th></th>
<th>Classical inverse method</th>
<th>Direct inverse method</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_1)</td>
<td>1.84 ± 0.01</td>
<td>1.83 ± 0.01</td>
</tr>
<tr>
<td>(H_2)</td>
<td>2.27 ± 0.02</td>
<td>2.28 ± 0.01</td>
</tr>
<tr>
<td>(K_1)</td>
<td>-0.018 ± 0.009</td>
<td>-0.017 ± 0.007</td>
</tr>
<tr>
<td>(K_2)</td>
<td>0.041 ± 0.008</td>
<td>0.043 ± 0.006</td>
</tr>
</tbody>
</table>

Table 7.3: Results of the classical and the direct inverse method using all runs at the same time. All optimizations were performed using the simplex algorithm.

required which introduced additional dispersion in the system that is not present in the adsorption profiles measured by the UV detector. This additional dispersion is inevitable and results in slightly longer tails in the chromatograms and hence in larger values for the dispersion coefficients.

7.5.3 Discussion

The novel direct inverse method has proven to be capable of estimating the adsorption isotherm parameters with the same accuracy as the classical inverse method. This result has two important implications: the first is the elimination of the conversion of a measured absorption value into a concentration value, i.e. elimination of calibration. The second is elimination of fraction analysis in the case of overlapping signals, which will save a significant amount of experimental work and resources.

A couple of remarks are worth making. First, the estimated parameter values do not depend on their initial guesses, whose choice influences at most the duration of the calculation. It is worth noting that the best estimate for \(K_1\) turned out always to be negative, whatever the sign of its initial guess. Second, the parameters were in our calculations unconstrained, i.e. there were no boundaries on the unknown parameters. However, it is possible to constrain the search in order to fulfill specific
7. The direct inverse method: a new method to estimate isotherm parameters

physical requirements. Third, the Henry constants are also estimated in this work through both the direct and classical inverse method. It is well-known however, that their values can be measured also through analytical pulse injections. The values for the Henry constants could then be fixed in the optimization problem, thus leaving the equilibrium constants and the dispersion coefficients only to be estimated and shortening the computation time.
Chapter 8

Concluding remarks

In the fine chemical and pharmaceutical industry, crystallization from solution is employed to produce crystalline particles with well-defined properties, e.g. polymorphic form, particle size, stability and purity. In this context, it is essential to elucidate the governing kinetic phenomena and to estimate the unknown parameters that appear in their expressions. Ultimately, accurate characterization of the different kinetic phenomena in crystallization, i.e. nucleation, growth and dissolution, allow process modeling and enable process design, optimization and control. This thesis aims at the quantitative application of in situ infrared and Raman spectroscopy to crystallization processes and at the determination of kinetic phenomena using these spectroscopic techniques.

Chapter 3 discusses different theoretical approaches that are applied throughout this thesis. This chapter focusses on different approaches to convert measured spectroscopic data into concentration values as well as on different approaches to describe crystallization processes using pop-
Chapter 4 addresses the quantitative application of in situ infrared and in situ Raman spectroscopy to different crystallization processes using a multivariate calibration-based approach. It was demonstrated that through variable selection and data preprocessing techniques, an accurate and robust estimation for the solute concentration was obtained even for a sparingly soluble substance such as L-glutamic acid in aqueous solution. It was shown that principal component analysis of Raman data enables fast end-point determination of L-glutamic acid’s solid-state transformation with little calibration efforts. An experimental design, covering process conditions that influence the Raman signal, has been proposed that allows for a quantitative application of Raman spectroscopy. The applicability of this approach for the quantification of the solid-state composition was shown for seeded and unseeded polymorph transformation experiments under different process conditions. Additionally, it was shown that multivariate data analysis enables also the monitoring of the solute concentration despite the fact that the solute signals in the Raman spectra are weak and completely overlapping with the signals associated to the solid phase.

In the first part of Chapter 5, it was demonstrated that the quantitative use of in situ Raman spectroscopy enables new insights in the fundamental mechanisms of L-glutamic acid’s polymorph transformation. In situ Raman spectroscopy was used to determine the secondary nucleation rate of the stable $\beta$ polymorph of L-glutamic acid at various operating conditions. Given that the growth rate of the stable $\beta$ polymorph was determined previously, the Raman spectra recorded during the transformation experiments were used together with a population balance model and a nonlinear optimization routine to estimate the secondary nucleation rate of the $\beta$ polymorph, which was otherwise not directly
accessible. From the experimental observations it was concluded that an attrition-based nucleation mechanism governs the transformation of the metastable $\alpha$ to the stable $\beta$ polymorph. For the first time, the effect of stirring on the course of a polymorph transformation was modeled using a population balance model and relevant kinetics. Finally, the proposed model allowed calculating the course of a polymorph transformation in a fully predictive manner for seeded as well as for unseeded conditions under a wide range of operating conditions. In the second part of Chapter 5, a novel approach to apply in situ Raman spectroscopy in a quantitative manner, avoiding the development of a calibration model, was presented. This approach fits the measured time-resolved Raman spectra directly and results in the concentrations of the different analytes as a function of time. The method has been discussed and tested thoroughly using synthetic as well as experimental Raman data acquired during the solvent-mediated polymorph transformation of L-glutamic acid. Using experimental Raman spectra it has been demonstrated that the modeled concentration profiles based on fitting the time-resolved Raman data show a good agreement with the estimated concentrations using a previously developed multivariate calibration model. Additionally, the estimated kinetic parameters based on fitting Raman spectra are very similar to those obtained through fitting the solid phase composition profiles obtained from the calibration model. Therefore, this approach enables quantitative understanding of a solid-state transformation process without the necessity of developing a calibration model and thereby saving a significant amount of time and resources.

Using seeded batch desupersaturation experiments and a population balance model, it was demonstrated in Chapter 6 that by fitting the measured time-resolved ATR-FTIR spectra directly and by fitting the desupersaturation profiles obtained from these spectra combined with
a previously developed calibration model, essentially the same crystal growth rate parameters for paracetamol in water can be estimated. Furthermore, the measured time-resolved Raman spectra were also fitted directly using the same population balance model and very similar crystal growth rate parameters could be determined.

A new method to estimate adsorption isotherm parameters using liquid chromatography, i.e. the direct inverse method, was presented in Chapter 7. This novel approach assumes a linear dependency of the UV absorption intensity on the solute concentration in the mobile phase and can easily be derived from the calibration-free approach presented earlier. It was demonstrated using synthetic as well as experimental data, that by fitting the absorption profiles, i.e. the direct inverse method, and by fitting the concentration profiles, i.e. the classical inverse method, very similar adsorption isotherm parameters can be estimated. This has two important implications: the first is the elimination of the conversion of a measured absorption value into a concentration value, i.e. elimination of calibration. The second is elimination of fraction analysis in the case of overlapping signals.

Thus summarizing, this thesis addresses the quantitative application of in situ spectroscopic techniques to crystallization from solution. When combining these in situ spectroscopic tools with detailed process modeling, relevant kinetics that govern crystallization processes can be estimated. On the one hand, classical calibration-based approaches allow measurement of the time-resolved concentrations that can then be used for process modeling. On the other hand, a novel calibration-free approach enables the estimation of kinetic parameters avoiding extensive calibration efforts. When applying the calibration-free approach to an industrial crystallization process, it would be beneficial to start validating the linearity between the spectroscopic signal and the concentration.
Then, easily accessible kinetics such as growth rates could be estimated using the calibration-free method. At a further stage, more complicated processes such as a solvent-mediated polymorph transformation could be characterized. Ultimately this kinetic information could be employed for control purposes.

A drawback of all in situ characterization techniques discussed in this thesis is the experimental effort that is required in order to apply them. By reducing these experimental efforts, the broader application of these techniques to an industrial environment will become more attractive. The following first two areas have potential to reduce the time-consuming and costly experimental work:

- In the case a detailed process model is not available, calibration seems to be the only way to apply in situ infrared and Raman spectroscopy in a quantitative manner. One could however invest in the development of calibration-free approaches in the case no process model is available. One of these approaches may be multivariate curve resolution, however its application to crystallization processes is yet to be demonstrated\textsuperscript{42,43,117,118}. Another very promising approach is the application of the Bayesian estimation theory combined with Markov Chain Monte Carlo (MCMC) methods that was used recently to study the transformation of calcium carbonate polymorphs using in situ Raman spectroscopy\textsuperscript{119–122}.

- In order to estimate accurate kinetics, a certain number of experiments is always required, however a proper design of experiments can reduce the number of experiments, and thereby the experimental effort, to a minimum without losing on the quality of the estimated kinetic parameters. Another approach to reduce the number of experiments in a parameter estimation study may be
8. Concluding remarks

the so-called incremental identification of kinetics, an approach that has been successfully applied in homogeneous reaction engineering\textsuperscript{123–125}.

- Ultimately, in situ measurement of the particle size distribution (PSD) provides a maximum amount of information that could be used to describe secondary phenomena such as crystal breakage and agglomeration. Whereas in situ spectroscopic techniques provide overall process variables such as the solute concentration, the particle size distribution would provide even more insight in crystallization processes. The focused beam reflectance measurement (FBRM) is the only particle size measurement technique that is capable of measuring in situ. A generally applicable method to interpret chord length distributions (CLDs) in terms of the underlying PSDs is yet to be developed. More sophisticated statistical data analysis techniques such as principal component analysis might open up the way to a true quantitative application of the FBRM and to monitor the time-evolution of the PSD\textsuperscript{30}.

- The applicability of the calibration-free approach was demonstrated in this thesis through its application to crystallization from solution and to liquid chromatography. In principle there are two prerequisites for this calibration-free approach: the first is the linearity between the measured spectroscopic signal and the concentration and the second is a mathematical model that describes the time-evolution of the process. There could be other areas where these two prerequisites are fulfilled and where the calibration-free approach can be of great value; for instance granulation, polymerization processes and other chromatographic processes.
References


References


[22] Caillet, A.; Puel, F.; Fevotte, G. In-line monitoring of partial and overall solid concentration during solvent-mediated phase transi-


# Appendix A

## List of symbols

### Roman symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>$a$</td>
<td>pure-analyte intensity</td>
<td>[-]</td>
</tr>
<tr>
<td>$b$</td>
<td>calibration factor</td>
<td>[-]</td>
</tr>
<tr>
<td>$c$</td>
<td>concentration</td>
<td>[kg m$^{-3}$]</td>
</tr>
<tr>
<td>$D$</td>
<td>dissolution rate</td>
<td>[m s$^{-1}$]</td>
</tr>
<tr>
<td>$D_{ap}$</td>
<td>apparent dispersion coefficient</td>
<td>[m$^2$ s$^{-1}$]</td>
</tr>
<tr>
<td>$e$</td>
<td>experimental error</td>
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<tr>
<td>$G$</td>
<td>growth rate</td>
<td>[m s$^{-1}$]</td>
</tr>
<tr>
<td>$H$</td>
<td>Henry coefficient</td>
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<tr>
<td>$J$</td>
<td>nucleation rate</td>
<td>[m$^{-3}$ s$^{-1}$]</td>
</tr>
<tr>
<td>$k$</td>
<td>empirical parameter</td>
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<tr>
<td>$k_v$</td>
<td>volume shape factor</td>
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<tr>
<td>$K$</td>
<td>equilibrium constant</td>
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</tr>
<tr>
<td>$L$</td>
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<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>(m)</td>
<td>mass of particles</td>
<td>([\text{kg m}^{-3}])</td>
</tr>
<tr>
<td>(n)</td>
<td>number density of particles</td>
<td>([\text{m}^{-4}])</td>
</tr>
<tr>
<td>(q)</td>
<td>stationary phase concentration</td>
<td>([\text{g L}^{-1}])</td>
</tr>
<tr>
<td>(S)</td>
<td>supersaturation</td>
<td>[-]</td>
</tr>
<tr>
<td>(t)</td>
<td>time</td>
<td>([\text{s}])</td>
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<tr>
<td>(u)</td>
<td>superficial velocity</td>
<td>([\text{m s}^{-1}])</td>
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<tr>
<td>(w)</td>
<td>weight fraction or percentage</td>
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<tr>
<td>(x)</td>
<td>spectral intensity</td>
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<tr>
<td>(z)</td>
<td>space coordinate</td>
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<thead>
<tr>
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<tr>
<td>(A)</td>
<td>pure-analyte spectra matrix</td>
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<tr>
<td>(B)</td>
<td>regression matrix</td>
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<tr>
<td>(C)</td>
<td>concentration matrix</td>
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<tr>
<td>(E)</td>
<td>matrix of experimental errors</td>
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</tr>
<tr>
<td>(P)</td>
<td>loadings matrix</td>
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</tr>
<tr>
<td>(R)</td>
<td>matrix of residuals</td>
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<tr>
<td>(T)</td>
<td>scores matrix</td>
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<tr>
<td>(W)</td>
<td>weights matrix</td>
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<tr>
<td>(X)</td>
<td>spectral matrix</td>
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**Greek symbols**

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
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<tr>
<td>(\bar{\epsilon})</td>
<td>mean specific power input</td>
<td>([\text{m}^2 \text{ s}^{-3}])</td>
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<tr>
<td>(\epsilon^*)</td>
<td>overall bed void fraction</td>
<td>[-]</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>wavenumber or Raman shift</td>
<td>([\text{cm}^{-1}])</td>
</tr>
<tr>
<td>(\rho)</td>
<td>crystal density</td>
<td>([\text{kg m}^{-3}])</td>
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<tr>
<td>(\mu_j)</td>
<td>(j^{th}) moment</td>
<td>([\text{m}^j])</td>
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# List of abbreviations

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>CLD</td>
<td>chord length distribution</td>
</tr>
<tr>
<td>FBRM</td>
<td>focused beam reflectance measurement</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>LV</td>
<td>latent variable</td>
</tr>
<tr>
<td>MCMC</td>
<td>Markov chain Monte Carlo</td>
</tr>
<tr>
<td>MCR</td>
<td>multivariate curve resolution</td>
</tr>
<tr>
<td>MLR</td>
<td>multiple linear regression</td>
</tr>
<tr>
<td>MSC</td>
<td>multiplicative scatter correction</td>
</tr>
<tr>
<td>ODE</td>
<td>ordinary differential equation</td>
</tr>
<tr>
<td>PAT</td>
<td>process analytical technology</td>
</tr>
<tr>
<td>PBE</td>
<td>population balance equation</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>principal component regression</td>
</tr>
<tr>
<td>PDE</td>
<td>partial differential equation</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>PLSR</td>
<td>Partial least squares regression</td>
</tr>
<tr>
<td>PSD</td>
<td>Particle size distribution</td>
</tr>
<tr>
<td>PVM</td>
<td>Particle vision and measurement</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-ray diffraction</td>
</tr>
<tr>
<td>RMSECV</td>
<td>Root-mean-square error of cross-validation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SNV</td>
<td>Standard normal variate</td>
</tr>
<tr>
<td>SSR</td>
<td>Sum of squared residuals</td>
</tr>
<tr>
<td>SVD</td>
<td>Singular value decomposition</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
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</table>
Appendix C

List of Figures

2.1 Experimental setup enabling simultaneous application of FBRM, Raman and ATR-FTIR spectroscopy. . . . . . . . 6

2.2 The principle of ATR spectroscopy. A ray of light is internally totally reflected off the surface of the probe window. The evanescent wave created in this way travels a short distance away from the window where molecules in solution absorb part of the light. . . . . . . . . . . . . . . . . . 8
2.3 Principle of Raman scattering. (a) Quanta of energy $h\nu_0$ interacts with the molecule (L-glutamic acid) resulting in inelastic scattering; (b) energy level diagram: irradiation with light quanta $h\nu_0$ may result in scattering of quanta with energy $h\nu^+_R = h\nu_0 + h\nu_s$ or $h\nu^-_R = h\nu_0 - h\nu_s$, anti-Stokes and Stokes scattering, respectively; (c) simplified Raman spectrum, signal at $\nu_0$ is due to Rayleigh scattering, signal at lower frequency (Stokes signal) has a higher intensity than the signal at higher frequency (Anti-Stokes signal).

2.4 The measurement principle of the FBRM. Particles in suspension backscatter the laser light emitted by the probe. The distance the light travels over the particle surface is the chord length.

3.1 The time-evolution of the first four moments (a) and the concentration (b) and the resulting particle size distribution at the end of the process (c).

4.1 Powder X-ray diffraction patterns of metastable $\alpha$ and stable $\beta$ polymorph, the insets show microscopy images of both polymorphs.

4.2 Influence of the solute concentration at 35°C (a) and of the temperature at 15.05 g/kg (b) on the infrared absorbance.

4.3 Temperature and concentration of calibration samples (○) used for ATR-FTIR monitoring and solubility curve (■) of L-glutamic acid. To enhance the visibility only half of the data points is displayed.
4.4 Influence on Raman spectroscopy of solid composition (a) at 2 wt.% of total suspension density and 25°C, the solute concentration was equal to the solubility of the α form except for the 100 wt.% β polymorph suspensions. Influence of the solute concentration on Raman spectroscopy at 25°C (b) for concentrations of 0, 21.7, 38.0, 43.4, 54.3, 65.1 g/kg. The arrow indicates an increasing solute concentration. .......................................................... 47

4.5 Particle size distribution of α crystals obtained by wet sieving. The displayed numbers indicate the size ranges of the sieves that were used. .......................................................... 48

4.6 Influence of particle size on Raman signal intensity in solid state (a) and suspension (b) indicated by the baseline corrected height of two characteristic peaks. All measurements were performed using α polymorph particles at 25°C and for the suspension measurements the suspension density was 2 wt.% and solute concentration was equal to the solubility of the α form. .......................................................... 49

4.7 Influence of the suspension density on the Raman signal intensity. The arrow indicates an increasing suspension density of β L-glutamic acid crystals at 25°C, (0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 wt.%). .......................................................... 49

4.8 Measured solubility of β L-glutamic acid in water (○) as function of time (a) and temperature (b). In (b) the (○) represent solubility measurement by ATR-FTIR\textsuperscript{58} and gravimetrically determined solubilities are shown as (□)\textsuperscript{74}. 55
4.9 Measured solubility curve for oxalic acid in water (a) and paracetamol in water (b). In both figures measured solubility using ATR-FTIR spectroscopy is compared with gravimetrically determined solubility data shown as (□). 55

4.10 Concentration (a) and desupersaturation profiles (b) with respect to the β polymorph of L-glutamic acid in water for different cooling rates. The suspension density at 50°C equalled 0.5 wt.% in all experiments. 56

4.11 Solute concentration during an unseeded polymorph transformation $S_\alpha = 3.0$ at 45°C (a) and evidence of solid interferences due to α crystals sticking on the ATR window (b). The arrows indicate the characteristic signals for solid material. 58

4.12 Time-resolved Raman spectra for a seeded (a) polymorphic transformation using 30 g/kg seeds with a characteristic size $d_{43}$ of 197.8 µm at 45°C and an unseeded (b) polymorphic transformation at 45°C with an initial supersaturation $S_\alpha = 2.5$. Scores over time and in a three dimensional representation for the seeded (c,e) and unseeded (d,f) polymorphic transformations. The numbers in (c) and (d) represent the variance captured in the particular principal component. The curved arrows in (e) and (f) indicate the direction of the transformation. 61

4.13 Seeded polymorph transformation experiment (a) using 30 g/kg seeds with a characteristic size $d_{43}$ of 197.8 µm at 45°C. Unseeded polymorph transformation experiment (b) at 45°C with an initial supersaturation with respect to the α polymorph equal to 2.5. 65
5.1 Particle size distributions of $\beta$ seeds used for growth rate experiments. The average particle sizes were $d_{43}(F_{\beta,1}) = 41 \, \mu m$ and $d_{43}(F_{\beta,2}) = 132 \, \mu m$. . . . . . . . . . . . . . . . . 74

5.2 Particle size distributions of $\alpha$ seeds obtained by wet sieving. The following nominal size ranges of the sieves were used: 125 - 250 $\mu m$ ($F_{\alpha,1}$ sieve fraction, $d_{43} = 188 \, \mu m$), 250 - 355 $\mu m$ ($F_{\alpha,2}$ sieve fraction, $d_{43} = 303 \, \mu m$), > 355 $\mu m$ ($F_{\alpha,3}$ sieve fraction, $d_{43} = 412 \, \mu m$). . . . . . . . . . . . . . . . . 75

5.3 Desupersaturation profiles for two different seed fractions $F_{\beta,1}$ (a) and $F_{\beta,2}$ (b) as given in Figure 5.1. Symbols: experimental data; Lines: simulation results. . . . . . . . . . . . . . . . . 83

5.4 Comparison of the growth rates of the $\alpha$ and the $\beta$ polymorph. Please note that the overall growth rate of a volume equivalent sphere is plotted for $\alpha$ and $\beta$, respectively. 84

5.5 Experimental repeatability of seeded transformation experiments in terms of solid composition (a) and liquid phase concentration (b). . . . . . . . . . . . . . . . . . . . . . . . . 85

5.6 Polymorph transformation with small seed crystals ($F_{\alpha,1}$ fraction). Symbols: experimental data; Lines: simulation results. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 86

5.7 Polymorph transformation with medium seed crystals ($F_{\alpha,2}$ fraction). Symbols: experimental data; Lines: simulation results. . . . . . . . . . . . . . . . . . . . . . . . . . 86

5.8 Polymorph transformation with large seed crystals ($F_{\alpha,3}$ fraction). Symbols: experimental data; Lines: simulation results. . . . . . . . . . . . . . . . . . . . . . . . . . 87
5.9 SEM micrographs showing $\alpha$ seeds contaminated with $\beta$ platelet particles. ......................................................... 88

5.10 Effect of stirring on seeded polymorph transformations. Symbols: experimental data; Lines: simulation results. ... 89

5.11 Effect of stirring on seeded polymorph transformations. Symbols: experimental data; Lines: simulation results. ... 89

5.12 Effect of initial amount of $\beta$ polymorph on transformation time. The initial concentration is given as mass percentage of the initial $\alpha$ seeds ($F_{\alpha,1}$ fraction with a concentration of 30 g/kg). Symbols: experimental data; Lines: simulation results. ................................. 93

5.13 (a) A representative unseeded transformation experiment with initial supersaturation $S_\alpha = 2.5$ where $t_i$ and $t_p$ represent induction time and transformation period, respectively. Lines represent simulation results and symbols experimental data. (b) Total transformation period ($t_i + t_p$), experimental (symbols) and simulated (line), as a function of the initial supersaturation. Experimental transformation times are the result of at least three transformation experiments and the variance is given as error bars in the plot. ......................................................... 95

5.14 Raman signal intensity at 25°C as a function of the solute concentration at 857 cm$^{-1}$ (filled symbols) and of the suspension density in the case of $\beta$ crystals at 865 cm$^{-1}$ (open symbols). ......................................................... 100

5.15 Simulated concentrations, matrix $\hat{C}$, (a) and the resulting simulated time-resolved Raman spectra (b) for an unseeded transformation with $S_\alpha = 2.25$ at 45°C. ................. 107
5.16 Fitting multivariate Raman data using 15 (parts a, c and e) and 50 (parts b, d and f) g/kg of the small seeds. The measured spectra acquired during the seeded transformations are shown in parts a and b. Parts c and d plot the measured and the fitted intensities at different Raman shifts. The modeled concentration profiles of the \( \alpha \) and the \( \beta \) form and the solute based on fitting the time-resolved Raman spectra directly are displayed in parts e and f. 

5.17 Comparison between the modeled concentration profiles based on fitting the time-resolved Raman spectra (lines) and the concentrations estimated using the multivariate calibration for 15 (a) and 50 (b) g/kg of the small sieve fraction. The solid phase composition profiles (symbols) and the predictions based on fitting the time-resolved Raman spectra (solid lines) and the predictions based on fitting the estimated solid phase composition profiles (dashed lines) in the case of the small (c) and the intermediate (d) sieve fraction using 15, 30 and 50 g/kg of seed crystals.

6.1 The integrated peak area (1200 – 1290 cm\(^{-1}\)) of paracetamol solutions in the case of ATR-FTIR spectroscopy (a). The integrated peak area in the case of paracetamol solutions (filled symbols, 1155 – 1185 cm\(^{-1}\)) and in the case of saturated suspensions (open symbols, 780 – 810 cm\(^{-1}\)) measured with Raman spectroscopy (b). All measurements were performed at 20\(^\circ\)C and the lines are a guide for the eye.
6.2 The measured particle size distribution of the seed crystals that were used in all batch desupersaturation experiments. The line indicates the fit of the measured points that was used in the population balance model and the average particle size $d_{43}$ equalled 156 $\mu$m.  

6.3 The repeatability of the performed experiments indicated by the measured time-resolved solute concentrations obtained through the recorded ATR-FTIR spectra combined with the developed calibration model (filled symbols). The solid concentrations (open symbols) were determined from the mass balance and the experiments were performed at 20°C and 200 rpm. 

6.4 The measured (symbols) and modeled (lines) desupersaturation profiles for the three runs. In all experiments, the temperature and stirring rate equalled 20°C and 200 rpm, respectively. 

6.5 The measured (filled symbols) and the simulated (line) particle size distributions at the end of run 3. The particle size distribution of the seed crystals (open symbols) is shown for comparison. The measured particle size distributions (symbols) result from Multisizer measurements and the simulated one (line) is obtained using the mathematical process model and the parameters reported in the first row of Table 6.1.
6.6 Fitting spectroscopic data in the case of ATR-FTIR spectroscopy (parts a, b, c and d) and Raman spectroscopy (parts e, f, g and h). In both cases, run 3 is analyzed. The time-resolved IR and Raman spectra are shown in the parts a and e, respectively. Parts b and f demonstrate the agreement between the measured (symbols) and the modeled (lines) intensities for IR and Raman spectroscopy, respectively. The estimated concentrations are shown in parts c and g. The resulting pure-analyte spectra (continuous lines) are compared to the measured ones (dashed lines) in the parts d and h for IR and Raman spectroscopy, respectively. . . . . . . . . . . . . . . . . . . . . . . . . . . 134

6.7 The estimated concentration profiles resulting from the three different modeling approaches (lines) and the measured concentrations (symbols) obtained from the calibration model applied to the IR spectra for run 3. . . . . . . 136

6.8 The estimated growth rates as a function of the supersaturation (continuous lines) at 20°C compared to the estimated growth rate at 16°C taken from the literature\textsuperscript{102} (dashed line). The growth rates are given in the range of supersaturation values that were used for the parameter estimation. It should be noted that the overall growth rate of a volume equivalent sphere is plotted for all cases. 138
7.1 UV intensity as a function of the solute concentration in the mobile phase in the case of phenetole (component 1) in part a and of 4-\textit{tert}-butylphenol (component 2) in part b, respectively. All measurements were performed at 22°C by injecting 5 mL of the solution without the installation of a column and the values shown are the values of the resulting plateaus.

7.2 The time-evolution of the concentrations (a), the pure-component spectra (b), the noise (c) and the resulting simulated UV elution spectra (d) used in the simulation studies in the case of Gaussian pure-component spectra.

7.3 The pure-component spectra (a) and the resulting simulated UV elution spectra (b) used in the simulation studies in the case of more realistic pure-component spectra. To show that the pure-component spectra are indeed different, the ratio of both has also been plotted (dashed line).

7.4 The absorption profiles for runs 1 to 3 (a) and for runs 4 to 6 (b).

7.5 The results of the classical and the direct inverse method in the case of run 1 (a and c) and run 4 (c and d). The intensities at wavenumbers 292 (highest intensity), 295 and 297 (lowest intensity) nm are plotted.

7.6 The results of the classical and the direct inverse method in the case of run 2 (a and c) and run 5 (c and d). The concentration values in the parts a and c were obtained using a fraction analysis. The intensities at wavenumbers 292 (highest intensity), 295 and 297 (lowest intensity) nm are plotted.
7.7 The results of the classical and the direct inverse method in the case of run 3 (a and c) and run 6 (c and d). The concentration values in the parts a and c were obtained using a fraction analysis. The intensities at wavenumbers 293 (highest intensity), 296 and 298 (lowest intensity) nm are plotted. .......................................................... 158

7.8 Estimated adsorption isotherms for both components using the classical (continuous lines) and the direct (dashed lines) inverse method. .......................................................... 160
Appendix D

List of Tables

4.1 Calibration set as used for the quantitative application of Raman spectroscopy. $w_\alpha$: fraction of $\alpha$ polymorph in total amount of solid; $w_s$: suspension density; $c_s$: solute concentration; $T$: temperature; $d_{43}^\alpha$, $d_{43}^\beta$: particle size of $\alpha$, $\beta$ polymorph respectively. $c_\alpha^*$ and $c_\beta^*$ indicate the solubility of the $\alpha$ and $\beta$ polymorph respectively. $w_{\alpha,\text{var}}$ indicates the different solid compositions: 0, 5, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 95, 100 wt.% of $\alpha$ polymorph. . . 51
4.2 Data analysis results of infrared spectroscopy. All data are mean-centered, each spectrum was smoothed using a moving average filter over 3 points and baseline corrected. The mean of the concentration used equalled 15.26 g/kg. LV: latent variable; RMSECV: root-mean-square error of cross-validation; $R^2$: correlation coefficient; $Q^2$: cross-validation coefficient. The * indicates that a scaled temperature has been incorporated as an additional variable.

4.3 Data analysis results of Raman spectroscopy. All data are mean-centered and data used for solute concentration quantification was corrected for an off-set. LV: latent variable; norm.: normalization; bsl: baseline; RMSECV: root-mean-square error of cross-validation; $R^2$: correlation coefficient; $Q^2$: cross-validation coefficient.

5.1 Experimental conditions of the seeded batch desupersaturation experiments at 45°C. Each experiment was repeated once.

5.2 Experimental conditions of the seeded transformation experiments at 45°C. Each experiment was repeated at least once.

5.3 Known mechanisms and their kinetics expressions at 45°C used in the population balance model.

5.4 Elucidated mechanisms and their kinetics expressions at 45°C used in the population balance model.

5.5 Mechanisms and their kinetics expressions at 45°C used in the detailed process model. A detailed discussion about the selection of the expressions and their parameters can be founded in the first part of this chapter.
5.6 Optimization results based on fitting the time-resolved Raman spectra compared to fitting the solid phase composition profiles using the three different sieve fractions. Parameters $k_1$ and $k_2$ constitute the secondary nucleation rate expression, $w_{\beta,i}$ represents the initial amount of impurities of $\beta$ in the seed crystals expressed as wt.% of total seed mass. All optimizations were performed using the simplex algorithm.

6.1 The estimated crystal growth rate parameters resulting from the three different modeling approaches applied to the three experiments described above. All optimizations were performed using the simplex algorithm.

7.1 Experimental conditions of the different runs. All experiments were performed at 22°C. The first element of the feed concentration $c_f$ represents the feed concentration of phenetole (component 1).

7.2 Results of the classical and the direct inverse method using three different sets of experimental data. All optimizations were performed using the simplex algorithm. The values for the dispersion coefficients are given in $10^{-5}$ m$^2$ s$^{-1}$. For reasons of clarity, the subscript $ap$ has been omitted for the dispersion coefficients.

7.3 Results of the classical and the direct inverse method using all runs at the same time. All optimizations were performed using the simplex algorithm.
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