Application of process analytical technology (PAT) tools for the better understanding and control of the crystallization of polymorphic and impure systems

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1. Summary of the work and key results

Crystallization is an important unit operation, used as separation and purification technique for more than 90% of the APIs (active pharmaceutical ingredients) on the market (1). Therefore, monitoring and control this process is fundamental to ensure the quality of most medicines. The use of process analytical technology (PAT) tools during the drug development stage (laboratory and pilot plant scales) has largely helped in better understanding and optimizing both batch and, more recently, continuous crystallization processes (2). However, further work is still needed for the successful implementation of such technologies at industrial scale (3,4), especially for polymorphic systems. Polymorphism is the capacity of a compound to crystallize in more than one different crystalline structure, which can have different properties such as density, melting point, bioavailability and solubility. Understanding this phenomenon as well as being able to monitor and control it during industrial crystallization is one the biggest challenges for pharmaceutical industries.

The PhD thesis summarized here presents a comprehensive study on the application of PAT tools to study, monitor and control polymorphism during batch cooling crystallization processes (5). It is shown how the same analytical techniques can be used for real-time, feedback control of the polymorphic purity of the solid crystals in batch crystallizers, as well as for fundamental studies of polymorphic systems. In particular, for the investigation of the relation between chemical equilibrium in solution and polymorphic outcome of cooling crystallization processes. The main contributions of the thesis are:

1) The determination of a good calibration practice (GCP) procedure for the application of quantitative Raman spectroscopy for monitoring polymorphic transformations. For the first time, a complete study on the effect of the different properties of the analysed sample on the Raman signal has been performed and used, together with design of experiments, to develop a calibration strategy for polymorphic form determination in slurries (**Chapter 4 and 5**).

2) The development of a novel active polymorphic feedback control (APFC) strategy that combines Raman and attenuated total reflectance (ATR) UV/Vis spectroscopy for the selection and growth of the stable form of an organic compound after nucleation of a mixture of polymorphs or erroneous seeding. Raman spectroscopy can detect the undesired polymorph and triggers a heating cycle that dissolves it. After elimination of the metastable form, supersaturation control using ATR-UV/Vis can grow the stable

form. Both a model-free (experimental) and a model–based APFC were developed in this thesis (**Chapter 6 and 7**).

3) A first example of composite sensor array is presented in this work. Different combinations of PAT tools were used to monitor a polymorphic transformation: the signals were merged together and analysed using principal component analysis in order to obtain more immediate and understandable information. A selection criterion using rotated loadings was also proposed for the selection or the best combination of sensors for the study of a specific system (**Chapter 8**).
4) A comprehensive study on polymorphism of a zwitterionic compound in different solvents and with the addition of a structurally related additive. Cooling crystallizations of ortho-aminobenzoic acid (OABA) were performed in different combinations of solvents and additive: specific relations between the polymorphic form nucleated and the chemical interactions between additive-solute and solvent-solute were found (**Chapter 9 and 10**).

The last two results chapters of the thesis (**Chapter 11 and 12**) shows a case study conducted in collaboration with Purdue University (United States) and Hebei Welcome Pharmaceutical Co., LTD (China). The crystallization of a highly impure biopharmaceutical compound (vitamin B12) was investigated using focused beam reflectance measurement (FBRM), ATR-UV/Vis spectroscopy and particle vision and measurement (PVM).

The following paragraphs will briefly contextualize and summarize the key results from chapters **4**, **6**, **8** and **9** of the presented PhD thesis. References to corresponding peer-reviewed journals are also given for further information on the methodology and experimental results.

2. Application of quantitative Raman spectroscopy for the monitoring of polymorphic transformation in crystallization processes using a good calibration practice (GCP) procedure

Raman spectroscopy is a form of vibrational spectroscopy based on inelastic scattering of monochromatic light, usually from a laser source. It can be used to study solid, liquid and gaseous materials without requiring sample preparation (6,7). Most organic molecules present clear and resolved peaks in Raman spectra, offering the possibility to analyse both the liquid (composition and concentration) and the solid phase (crystalline structure and polymorphic ratio). Significant work has been done for the calibration of Raman spectroscopy to monitor the presence and amount of solid polymorphs in suspensions, as well as the liquid concentration during crystallization processes (8,9). Nevertheless, a clear and systematic approach to Raman calibration is missing in the literature.

Chapter 4 and 5 of the presented thesis aim at developing a systematic strategy for Raman calibration to determine the polymorphic ratio of slurries during crystallization processes. OABA was chosen as model compound; suspensions of the stable form I and the metastable form II were studied. Since most APIs are produced by cooling crystallization the calibration strategy was designed for this type of process. All parameters that can change during a cooling crystallization were identified and analysed systematically. In

particular, the effect of crystal size, temperature, solute and solid concentration on Raman spectra were studied and used to design a set of calibration experiments for the quantification of the polymorphic purity of OABA in slurries. Univariate and multivariate calibration techniques were investigated using several pre-processing techniques to optimize the signal.

The results are combined in a systematic "good calibration practice (GCP) procedure", that allows obtaining high quality measurement with a reduced amount of experiments. The experimental work was published in a peer review journal (10). The procedure proposed is very general and can be applied to any other spectroscopic technique and process; its key points are:

1) The identification of the parameters that can vary during the process of interest;
2) The study of the effect of the identified parameters on the selected spectroscopic technique;
3) The determination of calibration experiments by design of experiment (DoE) using the most influencing parameters.

The experiments showed that solute and solid concentration strongly affect Raman spectra while crystal size and temperature cause only minor changes. Additionally, it was found that, because of the strong effect of solid density on the Raman signal, the estimation of the solid concentration of the metastable form II is more accurate than the estimation of the polymorphic ratio of the solid phase. The Box-Behnken Design of Experiments approach was used to define the experimental conditions of 15 calibration experiments considering, as factors, solute concentration, solid density and concentration of form I and II in the slurry. Partial least square (PLS) and principal components (PC) regressions were used to calculate a multivariate calibration function for the measurement of the concentration of the metastable form II in suspension during a cooling crystallization process. Figure 1 shows the results of both calibrations: a good linearity can be observed and confirmed by values of $R^2 > 0.99$ for both cases.

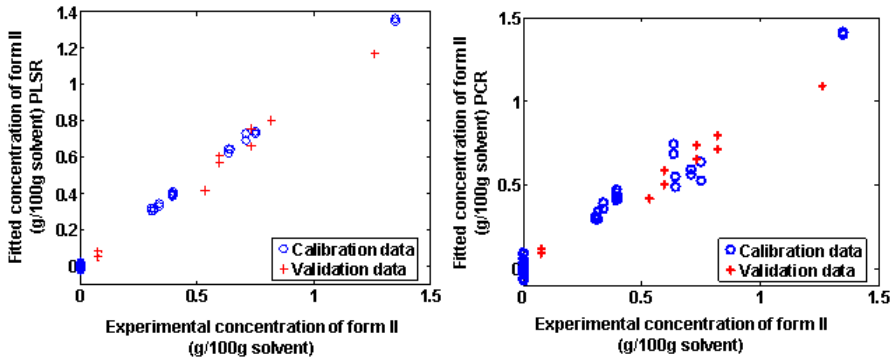

Figure 1: PLSR and PCR calibration and validation concentrations of form II with data obtained from the DoE.

The calculated root mean square error of prediction was found to be 0.04 g/100g of solvent for PLS regression and 0.06 g/100g solvent for the PC.

3. Active polymorphic feedback control of crystallization processes using a combined Raman and ATR-UV/Vis spectroscopy approach

Seeding with the desired polymorph is used most often in the literature as a way of controlling polymorphic purity during batch cooling crystallizations. Most strategies proposed use trial-and-error approaches (11,12), albeit greatly enhanced by the use of PAT tools, to design the operating trajectory that promotes the formation of the wanted polymorph, and only a few papers apply supersaturation control for direct feedback control (13–15). Most of these studies indicated that supersaturation control is an effective way to control the solute concentration and the growth of crystals as well as to reduce spontaneous nucleation of unwanted polymorphs; however it does not provide a suitable control approach alone, in the case of contaminated seed crystals or when the nucleation of unwanted polymorphic form does occur during the crystallization.

A different approach was recently proposed by Pataki *et al.* (16). In their work Raman spectroscopy was used to detect the presence of the metastable form of carvedilol. If such undesired polymorph was detected, the solution was heated up until complete dissolution and then cooled down with a different cooling rate to favor the nucleation of the stable polymorph. This approach in principle leads to the adaptive redesign of the operating conditions of the crystallization process by repeated recrystallizations of the product with decreasing cooling rate until the formation of the stable form is detected. It is not an actual feedback control technique, as it did not attempt to control the crystallization in the phase diagram directly, to avoid the formation of the unwanted form.

A novel active polymorphic feedback control strategy, based on the use of a combination of Raman and ATR-UV/Vis spectroscopy using a hierarchical control implementation is proposed in Chapter 6 of the thesis. The results were also published in a peer-review journal (17).

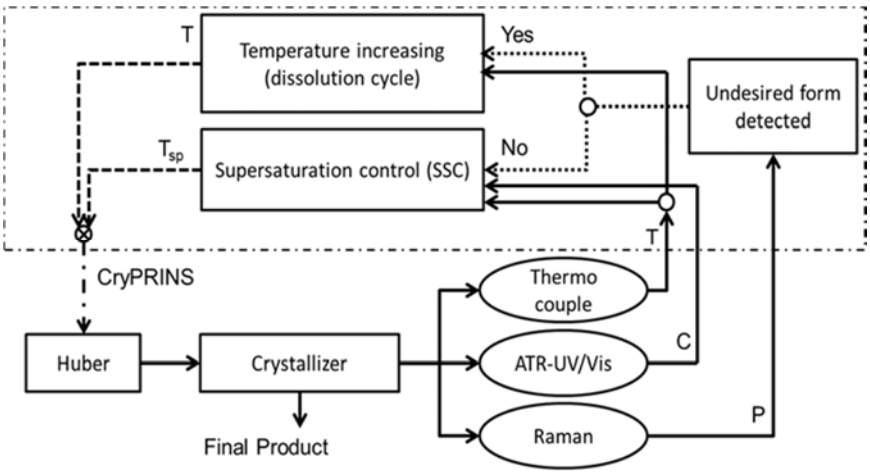

Figure 2: Schematic of the Active Polymorphic Feedback Control (APFC) approach. Raman spectroscopy is used to detect the presence of the undesired form (P) using a calibration-free approach; ATR-UV/Vis is used to measure the solute concentration (C) and perform SSC using a calibration-based approach to determine the setpoint for the temperature controller, (T_{sp}) (17).

In the APFC approach the formation of a polymorphic mixture is detected and the metastable form is eliminated by triggering a controlled dissolution cycle, after which the growth of the stable form is allowed using supersaturation control. A calibration-based approach is used to measure the solute concentration with ATR-UV/Vis for the supersaturation control; while for the Raman measurement a calibration-free technique is applied based on the identification of a specific peak in the spectrum associated with the presence of the metastable form. It has been shown that the proposed APFC technique can lead to pure polymorphic forms in the case of unseeded crystallization processes where nucleation of polymorphic mixtures occurs, or for crystallization processes erroneously seeded with a mixture of polymorphs. The APFC control method is presented schematically in Figure 2**Error! Reference source not found.**.

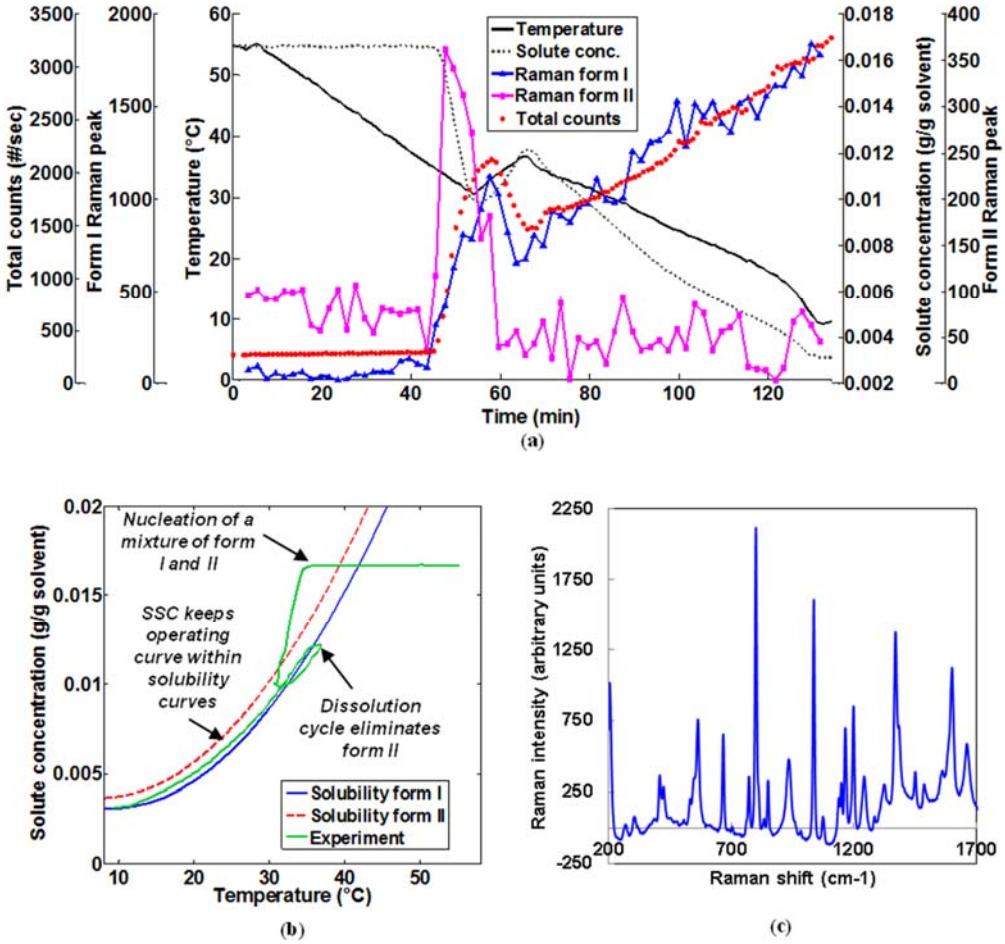

Figure 3: Results of the APFC approach applied to an unseeded experiment: (a) time evolution of temperature, concentration, Raman signals for form I and II and total counts/s from FBRM; (b) operating curve in the phase diagram; and (c) Raman spectrum for the final dried solid material confirming pure form I.

The Raman signal is used in the feedback control strategy to detect the presence of the polymorph contaminant and the active polymorphic feedback control (APFC) approach automatically triggers the dissolution cycle needed for its elimination both in the case of

seeded and unseeded systems. After the polymorph purity correction step based on the Raman signal, supersaturation control using ATR-UV/Vis spectroscopy is applied to maintain the operating curve in the phase diagram between the solubility curves of the stable and metastable polymorphs, hence avoiding any further contamination with the metastable form. The APFC approach was experimentally evaluated using OABA as the model system. The strategy was used to eliminate the metastable form II and grow the stable form I in both seeded and unseeded experiments. The results of an unseeded experiment performed on OABA are shown in Figure 3. A model-based active polymorphic feedback control (APFC) is presented in chapter 5 of the thesis. The kinetics parameters of OABA were estimated with a series of systematic experiments and then applied to model and optimize the APFC using the population balance equations solved with the method of moments.

4. In situ monitoring of polymorphic transformations using a composite sensor array of Raman, NIR, ATR-UV/Vis spectroscopy, FBRM and PVM, for an intelligent decision support system

The aims of chapter 8 of the thesis are to evaluate the capability of different PAT tools for monitoring polymorphic transformation during crystallization, to provide a systematic analysis of the signals and to investigate which combinations of tools is the best for the detection of various mechanisms in a particular system. Additionally, the framework of composite sensor array (CSA) is introduced, which is based on the integration of signals from multiple sensors using principal component analysis. Few examples of systems combining physically multiple pieces of hardware are present in the literature (18,19). However, none of them provided any mechanism for the integration of signals and data fusion. The concept of centralized CSA was first proposed by Nagy and Braatz (20), and is based on the integration of information from multiple PAT tools for automated detection of various events that may occur during crystallization. According to the concept of CSA, combining the signals from all the techniques is the same as having a single global sensor that can monitor all the different phenomena detectable by each individual tool. The signals can be analysed simultaneously using chemometrics techniques and combined in principal components that can more easily be interpreted and eventually used to control the process. Chapter 8 provides a systematic comparative investigation of the potential of different PAT tools in monitoring polymorphic transformations, and presents the first proof of concept of the CSA framework using chemometrics based data fusion. The chapter also introduces a methodology for the determination of optimal sensor configuration for the automated detection of various crystallization events. Experimental data were published in *Organic Process Research and Development* (21).
OABA was used in the experiments described in this thesis chapter. The system of PAT probes used included: (i) Raman; (ii) NIR transflection; (iii) NIR reflection; (iv) ATR-UV/Vis; (v) FBRM; (vi) PVM. Raman signal, total counts, and NIR or ATR-UV/Vis data were arranged in a single matrix after appropriate pre-processing. Principal component analysis (PCA) was then performed on such matrix.

Figure 4 shows the score of the first principal component (PC1) plotted versus the score of the second principal component (PC2) for four different unseeded crystallization experiments (the first three presenting a polymorphic transformation from OABA form II to I, while the last showing primary nucleation of the stable form I).

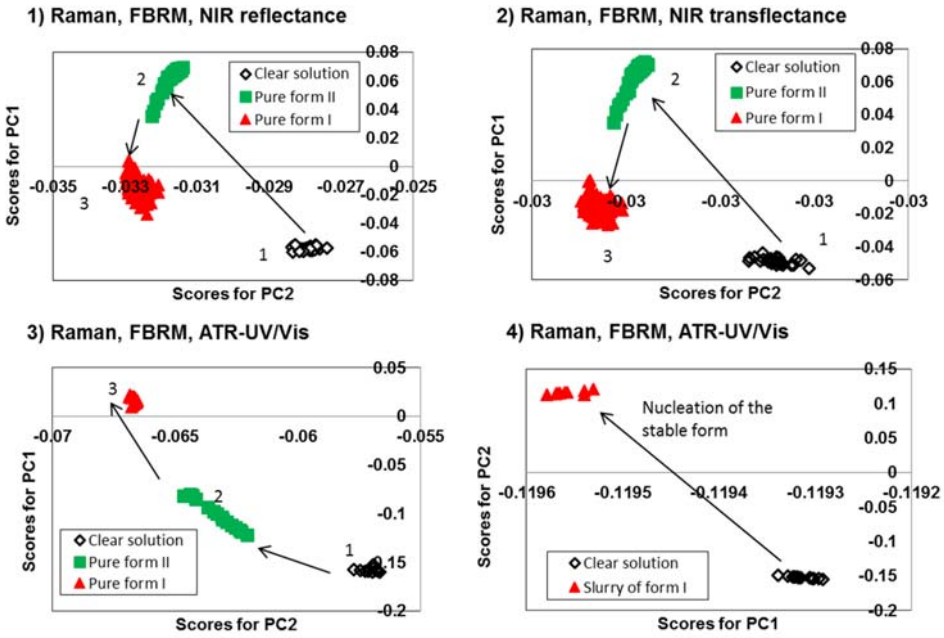

Figure 4: Plots of PC1 vs PC2 for clear solution, pure form I and pure form II in several experiments conducted using different collections of PAT tools. The graphs show the ease of detecting crystallization phenomena such as nucleation and polymorphic transformations.

Different groups of PAT tools were used in each experiment. Only points for pure form I, pure form II and the clear solution are represented in the plots in order to simplify the identification of the main clusters. The three species are clearly distinguishable in all the four plots. In particular, in Figure 4(1) (Raman + FBRM + NIR refl.) it can be notice that form I has a negative PC2 while form II has a positive one. The nucleation and transformation of one form could be easily identified in this system looking at the sign of PC2. For the CSA formed by Raman + FBRM +NIR transfl. a similar behaviour of the components can be noticed, as shown in Figure 4(2). In this case PC1 for form I is negative while is positive for form II. For the third type of CSA (Raman + FBRM + ATR-UV/Vis) PC2 for form I is positive and for form II negative, as shown in Figure 4(3). Plotting PC1 and PC2 together allows the immediate identification of the form nucleated or transformed.

PCA on the CSA formed by Raman, FBRM and ATR-UV/Vis was performed also in the case of nucleation of the stable form I (Figure 4(4)). Only two clusters can be identified for this last experiment: one for the clear solution and one for the solid form I.

Using more tools is useful to avoid misinterpretation of signals due to operating problems with a single probe (e.g. fouling and particle sticking in the case of FBRM and NIR of fluorescence for Raman). In this way the use of the CSA enables not only a better

understanding of the process, but also the identification of sensor failures or measurement problems. However, instead of always using a full CSA, identifying the best subset of PAT tools that should be included in the CSA to be able to monitor and identify particular combinations of crystallization mechanisms can save significant costs. In the final part of Chapter 8 of the presented thesis a methodology based on the rotated loadings of the principal components was developed to identify the sensors that affect most the PCs and help in the choice of an appropriate subset.

5. A link between the ATR-UV/Vis and Raman spectra of zwitterionic solutions and the polymorphic outcome in cooling crystallization

Understanding the solvent effect and how the equilibrium of the different chemical species in solution influences the nucleation and transformation of different polymorphs is fundamental for a better design and control of crystallization processes (22–24). Zwitterionic compounds represent an important class of active pharmaceutical ingredients; in the work described in chapter 9 of the thesis ATR-UV/Vis and Raman spectroscopy are used to investigate the relationship between the amount of zwitterions in the clear solution and the polymorphic outcome of cooling crystallizations, using the OABA as the model compound. The results were recently published in *Crystal Engineering Communications* (25). OABA can exist in three different polymorphic forms: I, II and III. The initial composition of the solvent, together with the supersaturation, can affect the nucleated form of OABA: under suitable conditions of solvent type and supersaturation it is possible to nucleate pure form I, form II or a mixture of form I and II (17). Form III is usually more difficult to nucleate or obtain through polymorphic conversion in solution below the transition temperature (around 50 °C).

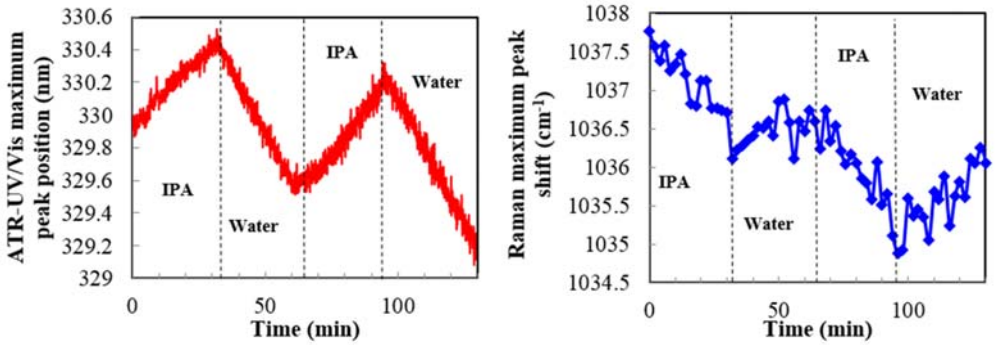

Figure 5: UV/Vis (a) and Raman (b) peak shifts during continuous alternate addition of water and IPA to an unsaturated solution of OABA in water (0.011 g OABA/g water). The flowrate was of 2 mL/min and water and IPA were pumped alternatively for about 30 min, twice.

A peculiar difference between solid form I and II of OABA is the presence of zwitterions in the crystal structure of form I, which are not present in form II. Zwitterions prevail over uncharged molecules in water (82% of all OABA molecules in aqueous solution), while organic solvents, such as iso-propyl alcohol, ethanol and acetonitrile favour the presence

of uncharged molecules. Changes in the ratio between zwitterions/uncharged molecules in solution are associated with shifts of specific OABA peaks in both the UV/Vis and the Raman spectrum as shown in Figure 5. The main UV/Vis peak of OABA has its maximum at 330 nm in water but shifts to higher wavelengths as the amount of zwitterions in solution decreases in favour of the uncharged molecules. The strong Raman peak associated to the OABA aromatic ring vibration (around 1037 cm^{-1}) shows an opposite but analogous trend. In order to find a relation between solution composition and polymorphic outcome, cooling crystallization experiments were performed using two different cooling rates (-1 and -0.5 ^{0}C/min), two level of saturation temperature (30 ^{0}C and 40 ^{0}C) and different solvent compositions (from 0 to 25% IPA in water). Both Raman and UV/Vis spectra of undersaturated solutions (before cooling) were collected and the position of the two specific peaks evaluated. Figure 6 shows the Raman and UV/Vis peak positions in undersaturated solutions at different solvent compositions together with the polymorphic outcome of the crystallization experiments. It can be observed that if the UV/Vis peak position is 331 nm or at higher wavelengths only form II is nucleated while below 328 nm only form I precipitates. A similar trend characterized the Raman peak: at shifts lower than 1036.5 cm^{-1} only form II nucleates while pure form I can be obtained if the peak is at shifts higher than 1037.5 cm^{-1}.

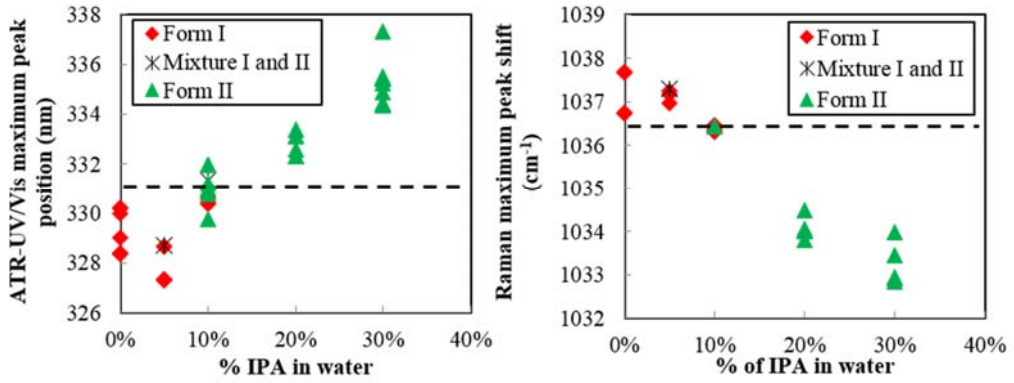

Figure 6: (a) UV/Vis and (b) Raman OABA peak positions in undersaturated conditions and corresponding polymorphic outcomes for cooling crystallization experiments. Two different cooling rates (-1 and -0.5 ^{0}C/min), two level of saturation temperature (30 and 40 ^{0}C) and several different solvent compositions (from 0 to 25% IPA in water) were used.

In conclusion, data show that the shift in the UV absorbance and in the Raman signal in clear solution, caused by changing the equilibrium of different species of OABA (by modifying the composition of the solvent), can be correlated with the polymorphic outcome of the crystallization experiments. These results provide new insight on the effect of equilibrium of the ionic, zwitterionic and neutral species in solution on the polymorphic outcome of crystallization and demonstrate how UV and Raman spectroscopy can be used to control the polymorphic crystallization of zwitterionic compounds by manipulating the solvent composition.

6. Importance and impact of the presented research

The work presented in the described PhD thesis focuses on the use of PAT tools for understanding, monitoring and control of polymorphic systems. It is shown how these technologies can be used for real-time feedback control and monitoring of crystallization processes as well as for fundamental understanding of polymorphism. The presented results provide useful information for both industries and other academics in the field of industrial and pharmaceutical crystallization.

The high quality of the work was recognized with four poster prizes at national and international conferences (ChemEngDay 2013, BIWIC 2013, EuroPACT 2014 and ISC 2014), over £3000 in travel grants to participate to conferences and fund further research collaborations and the selection for oral presentation at the main crystallization and process analytical technology conferences (BACG 2013, BIWIC 2014, ISIC20, BACG 2015 and EuroPACT 2017). Furthermore, the presented results resulted in 8 publications in high impact peer-review journals (7,10,17,21,26–29), which has already been cited over 80 times, as well as a section of a book chapter currently in preparation (Crystallization Process monitoring using PAT in "Process Systems Engineering for Pharmaceutical Manufacturing" published by ELSEVIER).

7. References

1. Nagy *et al.* (**2013**) *Chem Eng Res Des*. 91(10):1903–22.
2. Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacuring, and Quality Assurance. FDA Off Doc (2004).
3. Chen *et al.* (**2011**). *Cryst Growth Des*. 11:887–95.
4. Chieng et al. (**2011**). *J Pharmaceut Biomed*. 55(4):618–44.
5. Simone E. (**2015**). *Doctoral thesis*, Loughborough University, UK.
6. Févotte G. (**2007**). *Chem Eng Res Des*. 85(7):906–20.
7. Simone *et al.* (**2014**). *Chem Eng Technol*. 37(8):1305–13.
8. Wang *et al.* (**2000**). *Org Process Res Dev*. 4:391–5.
9. Cornel *et al.* (**2008**). *Ind Eng Chem Res*. 47:4870–82.
10. Simone *et al.* (**2014**). *Chem Eng Res Des*. 92(4):594–611.
11. Doki *et al.* (**2003**). *Cryst Growth Des*. 4(1):103–7.
12. Beckmann W. (**2000**). *Org Process Res Dev*. 4:372–83.
13. Doki *et al.* (**2004**). *Cryst Growth Des*. 4(5):949–53.
14. Kee *et al.* (**2009**). *Cryst Growth Des*. 9(7):3044–51.
15. Chew *et al.* (**2007**). *Cryst Eng Commun*. 9:128–30.
16. Pataki *et al.* (**2013**). *Org Process Res Dev*. 17 (3):493–499.
17. Simone *et al.* (**2014**). *Cryst Growth Des*. 14(4):1839–50.
18. Kadam *et al.* (**2012**). *Org Process Res Dev*. 16:769–80.
19. Helmdach *et al.* (**2013**). Org Process Res Dev. 17(3):585–98.
20. Nagy and Braatz (**2012**). *Annu Rev Chem Biomol Eng*. 3:55–75.
21. Simone *et al.* (**2015**). *Org Process Res Dev*. 19(1):167–77.
22. Kulkarni *et al.* (**2012**). *Chem Commun*. 48:4983–5.
23. Parveen *et al.* (**2005**). *Chem Commun*. 1531–3.
24. Davey *et al.* (**2013**). *Angew Rev*. 52:2166–79.
26. Simone and Nagy (**2015**). Crystengcomm. 17(34):6538–47.
27. Simone *et al.* (**2016**). *J Chem Technol Biotechnol*. 91: 1461-1470.
28. Simone *et al.* (**2015**). *Cryst Growth Des*. 15(6):2908–19.
29. Simone *et al.* (**2015**). *CrystEngComm*. 17:9370–9.