Available online at www.sciencedirect.com

SCIENCE DIRECT®

International Journal of Pharmaceutics 307 (2006) 201–208

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

In-line monitoring of partial and overall solid concentration during solvent-mediated phase transition using Raman spectroscopy

A. Caillet, F. Puel, G. Fevotte*

Laboratoire d'Automatique et de Génie des Procédés (LAGEP) UMR CNRS 5007, Université Claude Bernard Lyon 1,
ESCPE, Bât. 308G, 43 Bld, du 11 novembre 1918, F-69622 Villeurbanne Cedex, France

Received 6 October 2004; received in revised form 7 July 2005; accepted 3 October 2005
Available online 21 November 2005

Abstract

A calibration strategy for the continuous monitoring of solvent-mediated phase transition was developed using in situ Raman spectroscopy. Citric acid which exhibits enantiotropy during its anhydrous/monohydrate phase transition was selected as a model organic product. Using 25 samples in suspension, specific calibration of the spectral data was obtained for estimating in-line both the overall solid concentration in suspension and the composition of the solid phase. In addition to such key-measurements, reliable estimates of supersaturation were computed in-line from the mass balance of the solute. In order to validate the technique, anhydrous to monohydrate phase transition experiments were performed in suspensions at 15 °C and the kinetic process involved was monitored. Despite the use of various solid concentrations and reactor configurations, both the reproducibility and the reliability of the in situ Raman measurements are shown to be satisfactory.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Raman spectroscopy; Crystallization; Quantitative analysis; Solubility; Pseudo-polymorphism; Solvate; Transition kinetics

1. Introduction

In recent years, it has become obvious that the industrial production of solid drugs, in the near future will have to deal with new challenges including the control of parameters defining the quality of the particles: habits and size distribution, crystallinity, polymorphic state, chemical purity, etc. Meanwhile, it is well known that major limitations to improve the control of industrial crystallization processes arise from the lack of versatile, accurate and reliable in-line sensors. Most of the properties of the solid product, including therapeutic properties, are likely to depend on the solid phase, and among other quality variables, regulatory agencies advise the monitoring of the solid state of active pharmaceutical ingredients (API) in their guidelines, emphasizing the requirement for advanced analytical techniques. Moreover, from an economical point of view, as polymorphic forms are now considered to be an intellectual property, it is clear that nothing can be left to chance regarding the solid phases of the API. Moreover, it remains technically very difficult to estimate, even qualitatively, unexpected polymorphic changes during drug production, processing and storage. This is why both academic researchers and industrialists focus now an increasing part of their activity on the understanding, characterization, monitoring and control of possible polymorphic transitions during crystallization processes.

In the early stages of drug development, many analytical techniques are being currently used to characterise the crystalline form (e.g. Giron, 1995; Brittain, 1999; Yu et al., 1998). However, most of these techniques are unsuitable for the in-line monitoring of industrial processes leading to the generation of the solid product. Moreover, any manipulation of withdrawn solid suspension is extremely difficult to perform appropriately since one deal with metastable or fragile samples. This is why raising process analytical technologies (PAT) appear to be the most efficient and promising means of solving problems related to phase transformation phenomena during industrial solids elaboration processes. The Food and Drug Administration's PAT initiative is a significant illustration of the hopes which are put in the development of PATs. The major benefit expected from the use of PATs is to assure as high a quality as possible through timely measurements of critical quality and performance of raw materials, in-process materials and final products (Yu et al., 2004).

---

* Corresponding author. Tel.: +33 4 72 43 18 39; fax: +33 4 72 43 16 82.
E-mail address: fevotte@lagep.cpe.fr (G. Fevotte).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.10.009

Several spectroscopic PATs – namely attenuated total reflectance Fourier infrared spectroscopy (ATR FTIRS), near infrared spectroscopy (NIRS) and Raman spectroscopy – can be used to monitor in situ the state of solids generated during industrial crystallization processes. Among these techniques, Raman spectroscopy appeared in the past recent years as "one of the fastest, most reliable and most suitable techniques to identify crystals forms in drug products and can be easily exploited routinely for monitoring phase changes in drug products and quality control assays" (Auer et al., 2003). However, as far as continuous in-line and in situ measurements of the quality of the dispersed solid phase are concerned, few reported applications may be found in the open literature.

Using fiber-optic Raman spectroscopy, solute concentration was successfully monitored and controlled during the hanging-drop crystallization of lysozyme (Schwartz and Berglund, 1999, 2000; Schwartz et al., 2001). The concentrations of protein and salt ($(NH_4)_2SO_4$) were also measured during the hanging-drop crystallization of aprotinin (Tamagawa et al., 2002a,b). Agarwal and Berglund (2003) presented in situ Raman measurements of the polymorphic composition of calcium carbonate during its crystallization in the presence of various polymeric additives. The monotropic behaviour of progesterone was investigated by Wang et al. (2000) who measured the concentration of form I during the phase transition of form II into form I, according to Ostwald's Rule of Stage (ORS). A calibration curve was obtained relating the shift of an appropriate peak around 1665 cm^{-1} to the weight concentration of form I. The in situ monitoring allowed the authors to develop understanding of the process and define operating parameters allowing improved morphology of crystals to be obtained. In a similar spirit, Starbuck et al. (2002) used Raman spectroscopy to determine the rate of polymorphic transition of complex multipolymorphic a pharmaceutical compound, referred to as MK-A (polymorphs A, B, C, E and hemihydrate, dihydrate and NMP solvates were identified). In addition to the characterisation of such complex systems, Raman spectroscopy was useful in determining improved processing conditions. Moreover, the impact of potential disturbing events was investigated: inadvertent introduction of form C in the slurry and inadvertent water leakage into the solvent. The knowledge resulting from such studies is obviously invaluable for improving the industrial production process. More recently, the monitoring of antisolvent crystallization of progesterone was performed by Falcon and Berglund (2004) using in-line Raman spectroscopy. Principal component analysis (PCA) of the spectral data was shown to be efficient in detecting subtle features during the addition of antisolvent. Ono et al. (2004) monitored the polymorphic composition of L-glutamic acid in suspension during solvent-mediated phase transitions (i.e. α to β form). In order to measure the concentration of solid phases, the calibration of the Raman spectral data was performed from measurements of dry solid mixtures of the two polymorphs. The time variations of the concentration of the metastable α-form, which nucleates at 25 °C, provided kinetic information on its transformation into stable β-form.

The studies mentioned above have demonstrated the possibility of measuring the composition of polymorphic phases and of the solute concentration without considering explicitly the possibility of working with high and variable solids contents in the slurry. O'Sullivan et al. (2003) partly mentioned this major issue in the field of crystallization processes: for real industrial applications, the variations of specifications of the solid particles in suspension are likely to make any calibration difficult. This is why the present paper deals with the in situ monitoring of the phase transition of citric acid in suspension (anhydrous to monohydrate in water), which leads to highly concentrated media. As a first step, the goal here is to evaluate the possibility of measuring in-line both the overall solid concentration and the composition of the solid phase with various overall concentrations of solids in suspension.

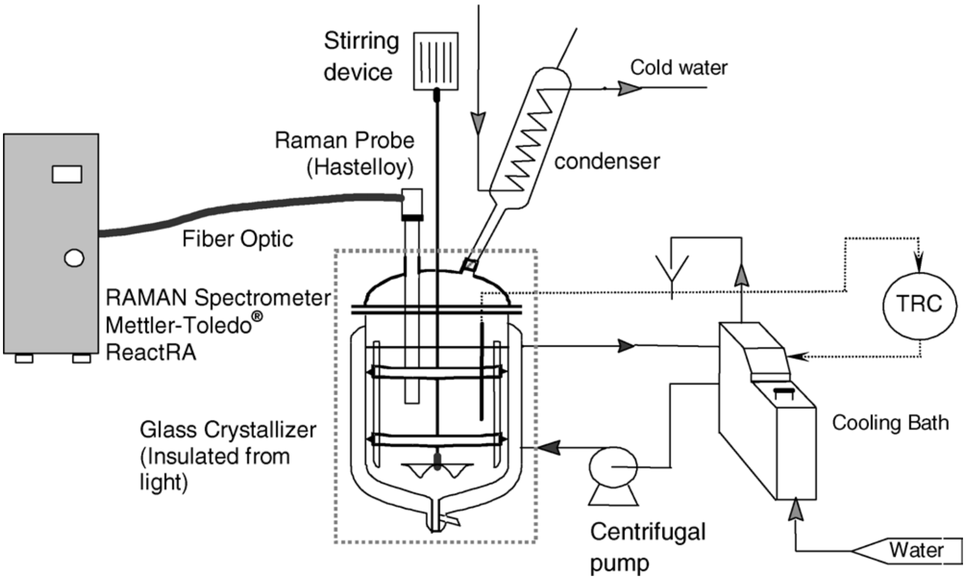

Fig. 1. Schematic of the lab-scale crystallization plant used for the Raman monitoring of solid phase transitions during solution crystallizations.

2. Experimental

Fig. 1 shows a schematic representation of the lab-scale crystallization equipment operated. A 250 mL glass reactor was used for most of the experiments presented below, and a 2.5 L glass reactor was used for other experiments where continuous in-line monitoring of the solid state was carried-out. Both glass vessels were equipped with a jacket and a condenser. Stainless-steel baffles were used in conjunction with a speed-controlled stirrer for the 2.5 L crystallizer. A high efficiency profiled propeller (Mixel TT^{TM}) maintained a good homogeneity of particles in the slurry. The whole operating device was instrumented and microcomputer-controlled in order to allow the tracking of temperature trajectories and/or of constant temperature setpoints.

In situ measurements were performed using the ReactRA Raman spectrometer manufactured by Mettler-Toledo, equipped with a 16 mm diameter immersion Hastelloy probe sealed with a sapphire window. As shown in Fig. 1 the probe is connected to the spectrometer through fiber-optics (200 μm) allowing spectral resolution of 7 cm^{-1}. The light source is an integrated stabilized 785 nm laser diode with 300 mW nominal output. The detection is ensured by an open electrode charge coupled device (CCD) with 1024 pixels × 256 pixels, cooled using a Peltier element.

The crystallization of citric acid in water was investigated as a model system. Data on the enantiotropic behaviour of the monohydrate/anhydrous transition of this product may be found in Groen and Roberts (2001). Fig. 2 shows that the solubility is very high (about 1.5 kg/kg water at 20 °C). The anhydrous is the more stable form above 34 °C while the monohydrate becomes more stable below this temperature. It should be mentioned that the solubility of the anhydrous at 15 °C was found to be slightly higher than that reported by Groen et al. (see Fig. 2). Both the hydrate and anhydrous solids were purchased from Acros Organics (citric acid anhydrous, reagent ACS, 99.5% and monohydrate p.a.), and used in distilled water without further purification.

3. Calibration procedure for the monitoring of phase transition of citric acid using Raman spectroscopy

In order to provide on-line estimates of the solid state, the Raman sensor requires a calibration model relating the variables of interest to the spectral measurements. However, such calibration should also take into account other process variables, such as temperature or overall solids content, which are known to have significant effects on the spectral data. Obviously, the dimensionality of the problem complicates the experimental procedure required. This is why one has to find a trade-off between substantial experimental work associated with refined multivariable models and reasonable experimental work associated with more specific models.

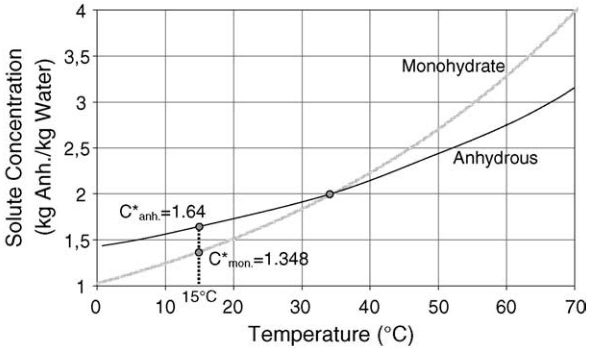

Fig. 2. Solubility curves of citric acid in water, the transition point is located at 34 °C (after Groen and Roberts, 2001).

The present study deals with isothermal experiments performed in slurries kept at 15 °C (i.e. anhydrous citric acid being metastable) under stirring. For calibration purposes, saturated solutions of monohydrate were prepared in the 250 mL crystallizer maintained at 15 °C. Twenty five mixtures of the two solid forms were then prepared to provide data with varying solid (5, 10, 15, 20 and 25 wt%) and anhydrous concentrations (0, 25, 50, 75 and 100 wt%) in suspension. The acquisition of Raman spectra was performed, assuming that during the first measurements no significant phase transition occurs. The concentrations were expressed as:

$$W_s = \frac{m_S}{m_S + m_L} \tag{1}$$

$$W_a = \frac{m_a}{m_S} \tag{2}$$

where m_S, m_L and m_a are the total mass of solid, the mass of solution and of anhydrous, respectively.

As explained below, the following definition of the concentration of total solid which converts the monohydrate present in the slurry to its equivalent in anhydrous was useful for calibration purposes:

$$\bar{W}_a = \frac{m_a + \frac{M_a}{M_m} m_m}{m_S + m_L} \tag{3}$$

where index m refers to the monohydrate solid form and M is the molecular weight of the corresponding compound.

3.1. Calibration of the measurement of anhydrous concentration W_a

Fig. 3 highlights the sensitivity of the Raman spectra to the solid state of the suspension between 1180 and 1070 cm^{-1}. Around 1080, 1105 and 1142 cm^{-1}, three maxima; denoted by a, b and c in Fig. 3, are identified as sensitive to the solution, the hydrate and the anhydrous variations, respectively. As in previously reported studies, correlations between the "peak heights" and the variables to be measured were sought. Several ways of expressing such correlations were investigated; keeping in mind that the measurement of relative heights is likely to reduce the sensitivity of the spectral data to undesirable variations (e.g. baseline fluctuations). One could expect usual chemometric techniques to provide satisfactory calibration model. Such techniques were rather satisfactorily developed but it finally appeared that the selected chemometric models were not in a position to ensure acceptable predictions for all calibration experiments. Several specific spectral variables were

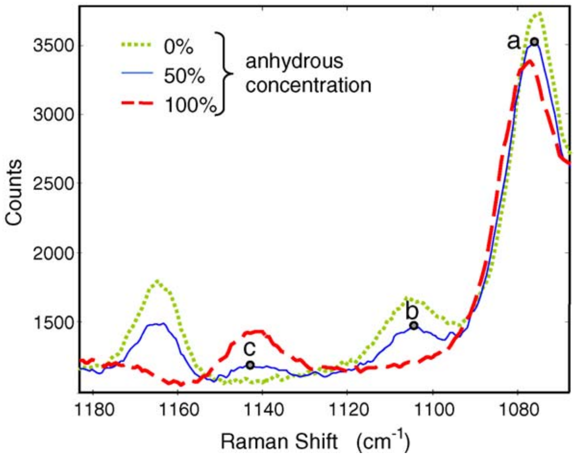

Fig. 3. Raman spectral region exhibiting sensitivity to the anhydrous concentration vs. monohydrate concentration of the solid phase.

then evaluated though cautious “trial and error” calibration procedures. The spectral variables in question were proposed so as to account for the spectral features exhibited in Fig. 3. The following relative parameter, Γ, which was computed from the calibration spectra, finally allowed us to obtain the “best” fit for the “measurement” of the anhydrous composition:

$$\Gamma = \frac{n_a - n_c}{(n_a - n_c) + (n_a - n_b)} \tag{4}$$

where n_a, n_b and n_c are the numbers of counts associated with points a, b and c, as represented in Fig. 3 for 50% anhydrous concentration.

Γ was then estimated as a function of the anhydrous concentration. Again, the mathematical expression of $\Gamma(W_a)$ was searched using a “trial and error” procedure: the experimental data were fitted to Γ through least squares minimization and the following function providing the best fit was selected:

$$\Gamma = p_1 \exp(-p_2 W_a) + p_3 {W_a}^2 + p_4 \tag{5}$$

with $p_1 = 0.454$, $p_2 = 1.43 \times 10^{-3}$, $p_3 = 3.42 \times 10^{-6}$ and $p_4 = -2.45 \times 10^{-4}$.

Expression (5) is plotted in Fig. 4 together with the calibration data. This kind of nonlinear relationship has already been presented in previous papers (Ono, 2004).

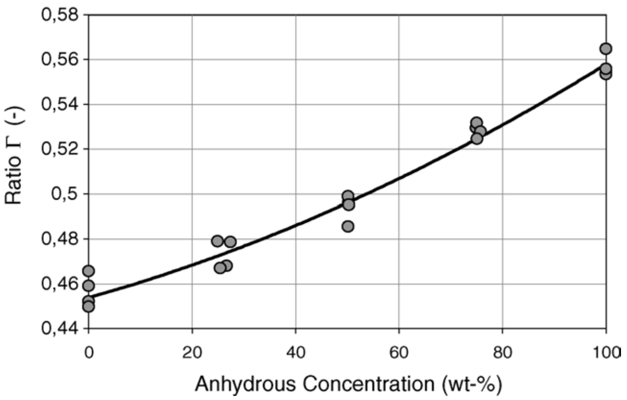

Fig. 4. Calibration curve fitting the ratio Γ defined by Eq. (5) as a function of W_a.

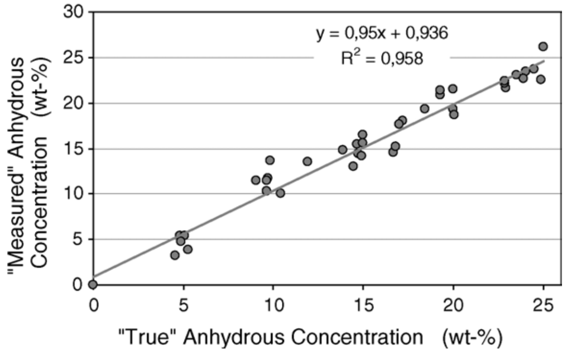

Fig. 5. Calibration cross-correlation plot of the measurement of $\bar{W}_S$. One “outlier” point was withdrawn.

3.2. Calibration of the measurement of the overall solid concentration $\bar{W}_s$

Several ways of operating the spectral data were assessed so as to increase the accuracy and reproducibility of the calibration model allowing estimation of the overall concentration of solid in suspension. Indeed, such a calibration turned out to be rather tricky to develop and any straightforward method, such as the fitting of appropriate peak heights to C_S failed. It finally turned out that improved consistency of the model predictions was obtained using $\bar{W}_S$, the concentration defined by Eq. (3).

Unlike the calibration of the measurements of the phase composition, poor results were obtained as one tried to relate simple spectral features of the Raman spectra to the overall solid content. Chemometric methods were then applied to the whole set of data. OPUS$^{\text{TM}}$ (Bruker Optics), which offers an extensive set of spectral processing routines, allowed the development of a satisfactory calibration model.

The whole set of spectral data was pre-processed through vectorial normalization and partial least squares (PLS) were then applied which led to the “best” estimation model. The calibration plot showing the estimated solid concentration (as defined by Eq. (5)) versus the actual concentration is given in Fig. 5. The computed root-mean square error is less than 2%, which was considered as satisfactory in the following. One data-point was identified as an “outlier” and withdrawn from the calibration data set.

3.3. Monitoring supersaturation

Assuming that the solubility data of the system in question are reliable, it is easy to compute supersaturation from the Raman measurements. The dimensionless solute concentration expressed in kg citric acid/kg water, is calculated according to the following equation:

$$C = \frac{\frac{C_0}{1+C_0} m_{L,0} + m_{a,0} - (m_L + m_S)\bar{W}_S}{\frac{1}{1+C_0} m_{L,0} - \frac{\bar{W}_S (m_L + m_S)(1 - W_a)\left(1 - \frac{W_a}{W_m}\right)}{W_a\left(1 - \frac{M_a}{M_m}\right) + \frac{M_a}{M_m}}} \tag{6}$$

where index 0 refers to the initial conditions. $m_{A,0}$ as previously defined, the mass of solid anhydrous introduced in the crystallizer and $m_{L,0}$ is the mass of initial saturated solution.

At 15 °C, the solubility of monohydrate and anhydrous citric acid in water were determined through titration: $C^*_{\text{mon.}} = 1.348$ kg/kg water and $C^*_{\text{anh.}} = 1.64$ kg/kg water.

In the following, the degree of supersaturation of monohydrate is therefore computed as (see Fig. 2):

$$\beta_{\text{mon.}} = C/C^*_{\text{mon.}} = C/1.348 \tag{7}$$

4. Results and discussion

As explained in introduction, our goal is to ensure a continuous monitoring of solvent-mediated phase transition process of the selected model system (i.e. citric acid in water). In order to assess the measurement technique we deal, in the sequel, with preliminary results showing some aspects of the dynamic features of isothermal anhydrous to monohydrate transitions performed at 15 °C.

4.1. Monitoring anhydrous to hydrate solvent-mediated phase transitions at 15 °C with varying initial solid contents

Saturated citric acid monohydrate solutions were prepared in distilled water at 15 °C, in the temperature-controlled 250 mL crystallizer described above. The stirring rate was set to 350 rpm. Commercial anhydrous citric acid was then added to the saturated solution, so as to obtain the following initial solid concentrations W_s: 15, 20 and 23.9%. Due to trace amounts (less than 0.5%) of monohydrate in the commercial anhydrous citric acid, spontaneous phase transition was observed during Runs 1–3 (see Table 1). The Raman spectra were subsequently recorded and processed, using the calibration models established previously. Here, the Raman acquisition was limited to 10–20 min. The phase transition process was not fully achieved as one can see in Figs. 7 and 8.

The measurements displayed in Figs. 6–8 are quite consistent with data expected from the operating conditions. The ratio of anhydrous citric acid decreases from 100% and tends to 0%. The initial value of the solid concentration is satisfactorily estimated and W_s tends to values in agreement with the final expected values computed from the solubility data (i.e. 18.8, 25.1, 29.9% monohydrate for the full transition of 15, 20 and 23.9% anhydrous, respectively). As shown in Fig. 8, the computed supersaturation profiles also present interesting features. The observed maximum was expected as a result of the two main competitive mechanisms involved during the transition: supersaturation first increases due to the dissolution of anhydrous and then decreases as a result of the growth of monohydrate crystals.

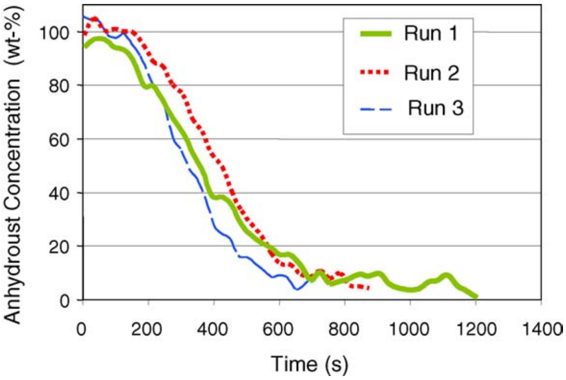

Fig. 6. Raman monitoring of the concentration of anhydrous citric acid during isothermal anhydrous to monohydrate transitions with various initial solid anhydrous content.

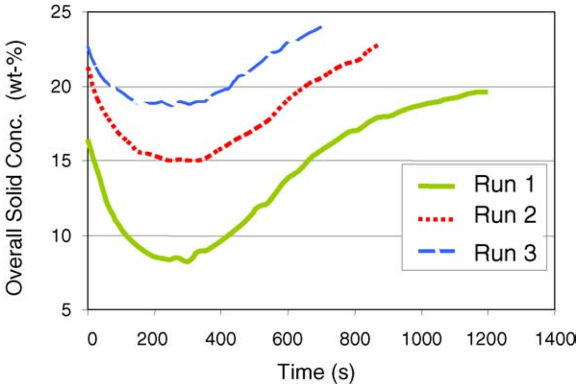

Fig. 7. Raman monitoring of the solid concentration W_s during isothermal anhydrous to hydrate transitions with various initial solid anhydrous content.

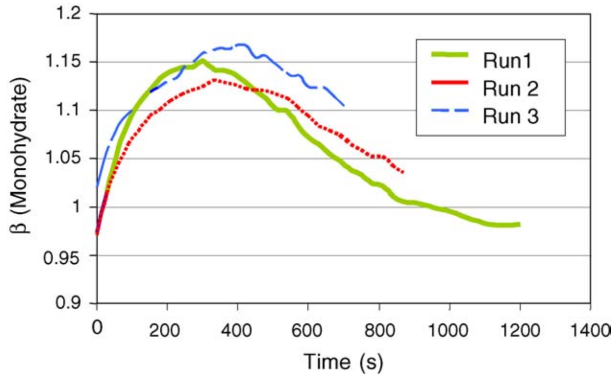

Fig. 8. Raman monitoring of isothermal transitions with various initial solid anhydrous content. Estimation of the supersaturation profile using the measurements in Figs. 6 and 7.

Table 1
Operating conditions for the lab-scale solvent-mediated phase transition experiments monitored using in situ Raman spectroscopy

Run no.	Initial solid anhydrous concentration (wt%)	Reactor volume (mL)	Stirring rate (rpm)	Temperature (°C)
1	15	250	350	15
2	20	250	350	15
3	23.9	250	350	15
4	20	250	350	15
5	20	2500	400	15

Even though this feature of the transition process will not be discussed in the present paper, it is also worth noting, from Fig. 6, that the overall rate of transformation of the anhydrous phase does not seem to depend significantly on the solids content.

Additional experiments (Runs 4 and 5, see Table 1) were performed, with similar operating conditions, but the acquisition was prolonged until the solvent-mediated phase transition was achieved. The two available reactors were used and the results were further compared. Such experiments allow, on the one hand, to assess the batch-to-batch reproducibility of the Raman measurements and, on the other hand, to evaluate the effect of the reactor configuration on both the rate of transition and the ability of the calibration to provide satisfactory measurement with different operating conditions. The results obtained with initial concentration $W_s = 20\%$ are presented below.

4.2. Validity of the calibration model for different reactor size

Fig. 9 displays the concentration of anhydrous during Run 2 and additional Runs 4 and 5. During Run 4 the operating conditions were identical to the conditions of Run 2, but the acquisition was prolonged for more than 2 h. Run 5 was performed with the same solid concentrations, but the 2.5 L reactor was used in this case.

The measurements of the anhydrous concentration during Runs 2 and 4 appear to be reproducible, while the rate of transition seems to be slightly increased during Run 5 performed in the 2.5 L glass reactor. When performed in the 250 mL crystallizer, the measurements of W_s present parallel trajectories with about 1.5–2% absolute uncertainty. The trajectory of the overall solid generation in the 2.5 L reactor presents a slightly different shape, which requires to be more deeply investigated in future experiments (see Fig. 10). However, given the relative uncertainty of the Raman measurements, one can consider that the quality of the estimates is satisfactory. The minimal and maximal values of the solid concentration are plotted in Fig. 10. The upper bound was calculated assuming full anhydrous to hydrate transformation, while the lower bounds, which can hardly be reached, corresponds to the hypothesis of total dissolution of the anhydrous citric acid together with negligible formation of monohydrate.

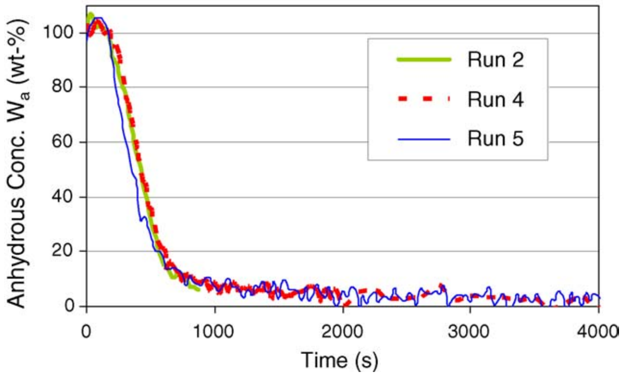

Fig. 9. Raman monitoring of the concentration of anhydrous citric acid during isothermal transitions performed with two different reactor sizes.

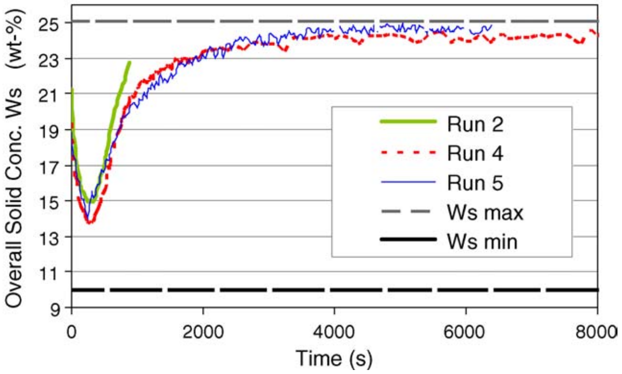

Fig. 10. Raman monitoring of the overall concentration of solid during isothermal transitions performed with two different reactor sizes. The minimal and maximal possible values for W_s are indicated.

At the beginning of the transition process, it is interesting to notice that the overall solid concentration decreases quickly, yet remains consistent with the lower bound $W_{s,min}$, the minimal value which can be estimated assuming that sufficient amount of anhydrous dissolves to reach the solubility of anhydrous at 15 °C (i.e. supersaturation is maximized with respect to monohydrate). This means that, compared with nucleation and growth of the monohydrate stable form, the rate of dissolution of anhydrous is high.

Runs 2, 4 and 5 allowed the evaluation of two important features which are related to the potential scaling-up of the experiments:

1. Given the complexity of the calibration, and the multivariate characteristics of the process, one could expect uncertainties to result from changes of any parameter related to the position of the Raman probe and/or the flow patterns described by the slurry in the two reactors used. Indeed, the differences observed between the measured concentration profiles, W_s and W_a, do not exceed the absolute uncertainty assessed after the calibration procedure. One can therefore consider that the calibration performed in the 250 mL reactor remains valid in the 2.5 L reactor. From a practical point of view and as far as scaling-up is concerned, this is a rather encouraging result.
2. It is an interesting industrial issue to evaluate possible differences that could result from scaling-up the kinetic parameters of phase transition phenomena estimated using in situ Raman measurements. As mentioned above, the differences presented by Run 5, compared with Runs 2 and 4, is very low. As one can see in Fig. 9, the time interval between to identical values of W_a, during the period of steepest descent, is about 80 s. Given the accuracy of the measurements and the unavoidable uncertainty about the beginning of the transition process, it is therefore difficult to conclude whether Run 5 is slightly faster than Runs 4 and 2, or not. One can therefore conclude that the size-dependency of the phase transition mechanisms, if the volume is increased by a volume factor of 10, is negligible.

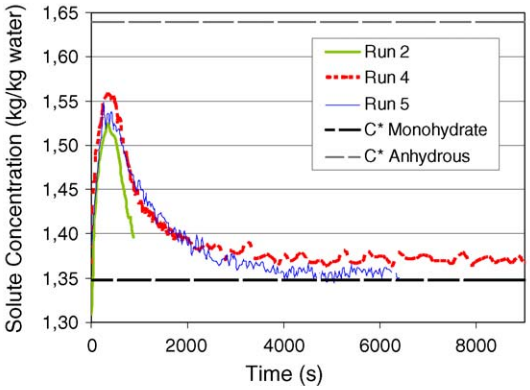

Fig. 11. Estimation of the solute concentration during the phase transition of citric acid using both in-line data displayed in Fig. 10 and this figure.

As far as the reliability of the in-line "predictions" is concerned, several points should be outlined:

- Even though this is not a sufficient condition, the estimates displayed in Figs. 6–11 always lie between their physical bounds: the anhydrous concentration evolves continuously from 100 to 0%. The degree of monohydrate supersaturation presents increasing and decreasing values starting from saturation (i.e. $\beta = 1$) and converging to final saturation (see Figs. 8 and 11). Such initial and final values of β were expected from the operating conditions since metastable solid was added to initial saturated monohydrate solutions. The same comment holds for the estimates of the overall solid concentration, as displayed in Fig. 10.
- Additional off-line measurements were performed from samples withdrawn from the 2.5 L reactor during further experiments. The estimates of the concentration of anhydrous citric acid were satisfactorily compared with DSC measurements (Caillet et al., 2005). The estimates of the dissolved solute concentration were also found to fit with "standard" titration measurements (Caillet et al., 2005), thus providing additional validation of the Raman calibration. However, for the sake of conciseness the complete experimental validation procedure is not reported in the present paper.

5. Conclusions

This work shows the great interest of in situ Raman spectroscopy as a process analytical technology for monitoring phase transition phenomena during industrial organic solution crystallization. Complete information about the solid phase (i.e. partial and overall solid concentration) was obtained in real time, which is a very significant advantage of the technique. Moreover, such information was shown to allow computing reliable additional estimates of supersaturation. The concentration of all the phases present in the slurry – three phases in the present study – are therefore accessible to measurements, allowing advanced monitoring of suspension crystallization processes to be developed.

The dynamics of solvent-mediated phase transition of citric acid was investigated as model system. As a first step, the study was restricted to isothermal conditions with varying initial overall solid concentrations. The experiments were performed in two different crystallizer volumes. The kinetics of phase transition, from the anhydrous to the monohydrate citric acid, were not found to depend significantly on the concentration of solid, nor on a scaling-up factor of 10. Despite obvious sensitivity of the measurements to the crystal size distribution, no specific investigation of the effect of this parameter on the reproducibility of the measurements was attempted. It should also be noted that temperature effects still have to be investigated. With flexible and reliable industrial applications of Raman spectrometry in view, taking these two parameters into account remains an important issue.

Acknowledgments

We are very grateful to our partners from Mettler-Toledo AutoChem Inc., for their significant help, and for the invaluable collaboration which was established between our laboratory and both the American and French teams involved in the development and marketing of the Mettler-Toledo ReactRA equipment.

References

Agarwal, P., Berglund, K.A., 2003. In situ monitoring of calcium carbonate polymorphs during batch crystallization in the presence of polymeric additives using Raman spectroscopy. Cryst. Growth Des. 3, 941–946.

Auer, M.E., Griesser, U.J., Sawatzki, J., 2003. Qualitative and quantitative study of polymorphic forms in drug formulations by near infrared FT–Raman spectroscopy. J. Mol. Struct. 661–662, 307–317.

Brittain, H.G., 1999. Polymorphism in Pharmaceutical Solids. Marcel Dekker, New York.

Caillet, A., Puel, F., Févotte, G., 2005. Raman monitoring of the phase transition of citric acid in water. Preprint of the 16th International Symposium of Industrial Crystallization ISIC'16, Dresden, 11–14 September 2005.

Falcon, J.A., Berglund, K.A., 2004. In situ monitoring of antisolvent addition crystallization with principal components analysis of Raman spectra. Cryst. Growth Des. 4, 457–463.

Giron, D., 1995. Thermal analysis and calorimetric methods in the characterization of polymorphs and solvates. Thermochim. Acta 248, 1–59.

Groen, H., Roberts, K.J., 2001. Nucleation, growth, and pseudo-polymorphic behavior of citric acid as monitored in situ by attenuated total reflection Fourier transform infrared spectroscopy. J. Phys. Chem. B 105, 10723–10730.

Ono, T., ter Horst, J.H., Jansens, P.J., 2004. Quantitative measurement of the polymorphic transformation of L-glutamic acid using in-situ Raman spectroscopy. Cryst. Growth Des. 4, 465–469.

O'Sullivan, B., Barrett, P., Hsiao, G., Carr, A., Glennon, B., 2003. In situ monitoring of polymorphic transitions. Org. Process Res. Dev. 7, 977–982.

Schwartz, A.M., Berglund, K.A., 1999. The use of Raman spectroscopy for in situ monitoring of lysozyme concentration during crystallization in a hanging drop. J. Cryst. Growth 203, 599–603.

Schwartz, A.M., Berglund, K.A., 2000. In situ monitoring and control of lysozyme concentration during crystallization in a hanging drop. J. Cryst. Growth 210, 753–760.

Schwartz, A.M., Berglund, Kris, A., 2001. Comparison of supersaturation profiles employed on lysozyme crystallization from a hanging drop. Cryst. Growth Des. 1, 81–85.

Starbuck, C., Spartalis, A., Wai, L., Wang, J., Fernandez, P., Lindemann, C.M., Zhou, G.X., Ge, Z., 2002. Process optimization of complex pharmaceutical polymorphic system via in situ Raman spectroscopy. Cryst. Growth Des. 2, 515–522.

Tamagawa, R.E., Miranda, E.A., Berglund, K.A., 2002a. Raman spectroscopic monitoring and control of aprotinin supersaturation in hanging-drop crystallization. Cryst. Growth Des. 2, 263–267.

Tamagawa, R.E., Miranda, E.A., Berglund, K.A., 2002b. Simultaneous monitoring of protein and $(NH_4)_2SO_4$ concentrations in aprotinin hanging-drop crystallization using Raman spectroscopy. Cryst. Growth Des. 2, 511–514.

Wang, F., Wachter, J.A., Antosz, F.J., Berglund, K.A., 2000. An investigation of solvent-mediated polymorphic transformation of progesterone using in situ Raman spectroscopy. Org. Process Res. Dev. 4, 391–395.

Yu, L., Reutzel, S.M., Stephenson, G.A., 1998. Physical characterization of polymorphic drugs: an integrated characterization strategy. Sci. Pharm. 1, 118–127.

Yu, L.X., Lionberger, R.A., Raw, A.S., D’Costa, R., Wu, H., Hussain, A.S., 2004. Applications of process analytical technology to crystallization processes. Adv. Drug Deliv. Rev. 56, 349–369.