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USING PROCESS ANALYTICAL TECHNOLOGY FOR IN SITU MONITORING OF THE POLYMORPHIC TRANSFORMATION OF ORGANIC COMPOUNDS

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Abstract: The paper presents a comprehensive evaluation study into the use of process analytical technology (PAT) for the *in situ* detection and monitoring of the polymorphic transformations from anhydrous to hydrate form, of a model system, caffeine in water. Techniques investigated include focused beam reflectance measurement (FBRM), on-line particle vision and measurement (PVM) and attenuated total reflectance (ATR) UV-spectroscopy. The influence of temperature and agitator speed on the hydration kinetics is evaluated. Results of the investigation into the use of PAT for the automatic metastable zone width determination and thermodynamic and kinetic study of the crystallization of caffeine are also presented. The enthalpy and entropy of caffeine dissolution and nucleation kinetics are reported.

Keywords: process analytical technology, pharmaceutical crystallization, focused beam reflectance measurement, attenuated total reflectance UV/Vis spectroscopy, particle vision and measurement.

1. INTRODUCTION

A key concern of the pharmaceutical industry is to maximize production efficiency while improving consistency and quality of the final products. Because many drugs are produced and marketed in the crystalline solid state for stability and convenience of handling, developments in the governing and regulating of crystallization have generated much interests in recent years. Polymorphism can be a major cause of significant variability in product performance in the pharmaceutical, chemical and food industry and continues to pose a challenge to crystallization scientists in producing products of consistent quality. However, polymorphism also provides a unique opportunity to engineer solids having tailor made properties (Stahl, 1980). Governed by these reasons, in recent years, the pharmaceutical industry has increasingly focused its attention on polymorphs. Pharmaceuticals can exist in various solid forms, including "real" polymorphs, solvates, hydrates or amorphous solids, having different physical and chemical properties. A significant number of pharmaceuticals exhibit the phenomenon of polymorphism; for example 70% of barbiturates, 60% of sulfonamides and 23% of steroids exist in different polymorphic forms (Stahl, 1980). Polymorphism can be the major cause of significant variability in product performance in the pharmaceutical, chemical and food industry and continues to pose a challenge to crystallization scientists in producing products of consistent quality. However polymorphism also provides a unique opportunity to engineer solids having tailor made properties (Stahl, 1980). Demands for improving pharmaceutical productivity and quality, as well as the encouragement of recent process analytical

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technology (PAT) initiatives have caused PAT to become increasingly embraced by pharmaceutical companies in both research and manufacturing. PAT has been recognized as a key tool in understanding and designing crystallization processes (Yu et al, 2004; Birch et al, 2005).

The paper presents an evaluation study of the crystallization of caffeine under different operating conditions. Caffeine is a pharmaceutically related molecule with three known forms; two anhydrous polymorphs and a hydrate (Edwards, et al, 1997). The hydrate is a channel hydrate that readily dehydrates to the anhydrous form. The anhydrous and hydrated forms of caffeine grow as crystals with very fine needle morphologies and from an industrial perspective this morphology often leads to issues with large scale crystallization and isolation. The use of various methods of PAT, including focused beam reflectance measurement (FBRM), on-line particle vision and measurement (PVM) and attenuated total reflectance (ATR)-UV-spectroscopy has been investigated, for the *in situ* detection of the caffeine hydration. The aforementioned PAT techniques have been used for the automatic determination of metastable zone width. The dissolution enthalpy and entropy as well as the nucleation kinetics are determined using the experimental data.

2. MATERIALS AND EQUIPMENT

Anhydrous caffeine from SigmaAldrich with a minimum purity of 98%, from the same batch were used for all samples, as a model system. Deionised water and solvents of laboratory grade were used in all tests. Experiments were performed at several scales in different systems. The process analytical technology used consists of: FBRM, ATR-UV/VIS and PVM, which were simultaneously used to monitor the system. The experimental setup is shown schematically on Figure 1.

Experiments at 1 L scale were performed in a Mettler-Toledo LabMax system, a computer controlled automated lab reactor (Mettler-Toledo Autochem Ltd, Leicester, UK). Various experiments were carried out in which solutions of caffeine in water were heated and cooled at different ramp rates, with a variety of stirring speeds. The samples were dosed with additional water after each run to change the concentration and allow solubility and MSZW measurements to be obtained. A D600l-HC-K FBRM Lasentec Probe (Mettler-Toledo Autochem Ltd, Leicester, UK) was used to detect the clear and cloud points in the solubility measurements. The data were collected every 20s in the 1-1000µm, 90 log channels mode. In certain experiments a Zeiss MCS 500 spectrometer with a Hellma 661.812 Attenuated Total Reflection (ATR) UV/Vis probe (supplied by Clairet Scientific, Northampton, UK) was used to collect information about the change of absorbance of caffeine in water as it was heated and cooled. The temperature was maintained using a Huber Ministat unit (Radleys Ltd, Saffron Waldon, Essex, UK), which was controlled with a PC. Crystallisation was observed using a particle vision monitor (PVM, Mettler-Toledo Autochem Ltd., Leicester, UK), and the FBRM probe used was the same as on the 1L scale. Additionally, experiments on a 500ml scale jacketed vessel were also performed, using an FBRM (A100, Lasentec) probe, and a stainless steel 4-blade turbine agitator, in a fully computer controlled system, using a fieldpoint interface and software developed in Labview (national Instruments, US). An Olympus BX51 microscope (Olympus UK Ltd, Southall, UK) with a 3-CCD colour video camera (JVC model KY-F55B) were used for microscopic examination of the crystals. Images were analysed using ImagePro Plus image analysis software (Media Cybernetics Inc, Silver Springs, USA).

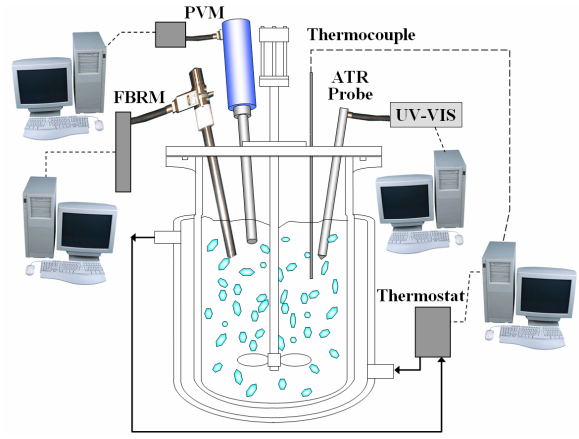

Fig. 1. Schematic representation of the equipment.

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1 Study of the polymorphic transformation using PAT

The first objective of the experiments was to evaluate how various PAT (e.g. PVM, FBRM and ATR-UV/Vis) approaches can be used for *in situ* detection of the polymorphic transformation. Undesired transformations can create difficulties during mixing, handling and filtration. Therefore the on-line detection of changes in the polymorphic form, can lead to the determination or correction of operating conditions that avoid later consequences (e.g. product degradation due to non-uniform mixing, blockage of pipes, excessive filtration time, etc.). The capability of the aforementioned PAT tools, was tested to detected the polymorphic transformation in the caffeine-water system. Although in the water system the hydration of caffeine is natural to occur, in practice often mixtures of water and organic solvents are used, when the exact operating conditions (e.g. solvent ratio, temperature or agitation) and the moment when this phenomenon occurs are not well defined, thus the in-situ detection of the polymorphic transformation is of significant practical importance. The transformation of the anhydrous form of caffeine into the hydrate is characterised by a significant change in the crystal shape, from the irregularly

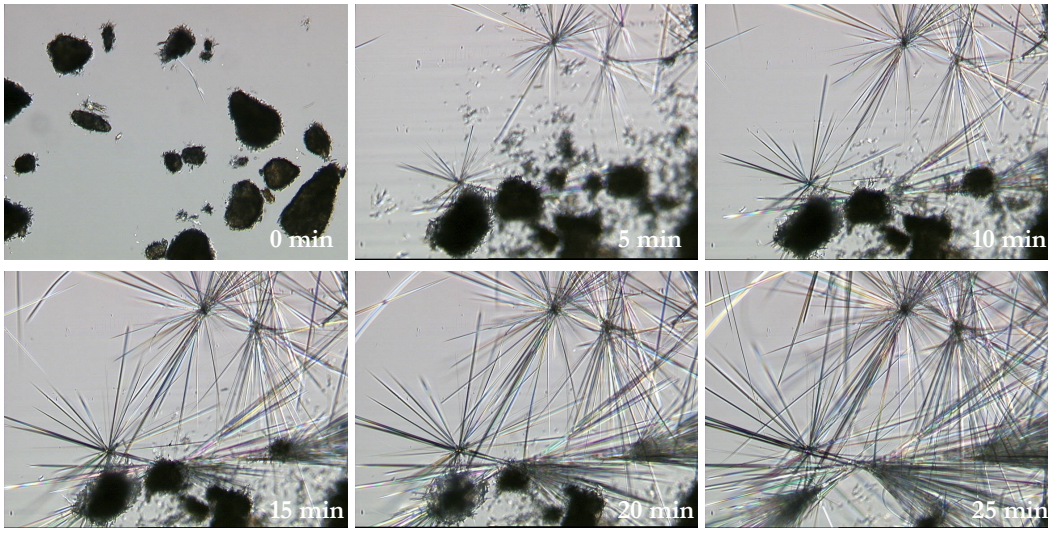

Fig. 2. Caffeine in Water. Sequence of microscopic images, illustrating the time evolution of the transformation process of anhydrous caffeine into caffeine hydrate.

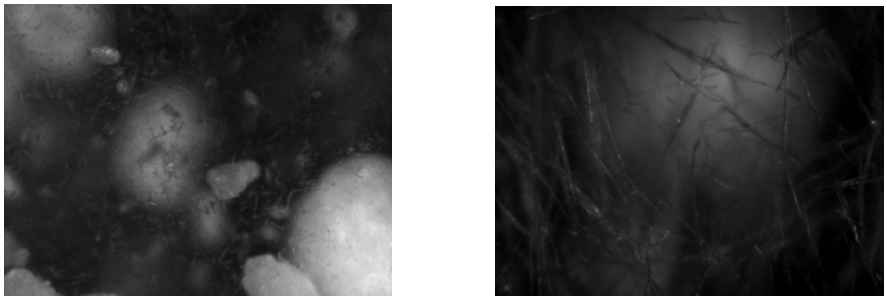

Fig. 3: PVM images obtained during crystallization of caffeine in water: (left) anhydrous caffeine; (right) after hydration started.

shaped powder particles of the anhydrous form to long needles for the hydrate. The transitions was studied under optical microscope, by adding water drops to the anhydrous caffeine powder and recording the transformation at room temperature and without agitation. Figure 2, shows a time sequence of images, which illustrates the polymorphic transformation.

Particle vision measurement (PVM) can provide an *in situ* observation of the polymorphic transformation that occurs during the formation of the hydrate. Figure 3 shows the PVM images before and after the hydration of caffeine, indicating the clear difference between the morphologies of the anhydrous and hydrated forms. Using the PVM probe the transformation can be studied under real process conditions. Currently an approach is being developed that uses image analysis techniques to identify the polymorphic change automatically. An automatic line detection approach is used to identify the needle shaped caffeine hydrate crystals and multivariate statistical analysis is performed to identify the change in the information content of the images.
Due to the existence of chromophoric group in the caffeine molecule it absorbs UV lights. The UV spectra of caffeine has two characteristic peaks (at 205 and 274 nm) and the absorbance at these wavelengths can be used to monitor the concentration change in the solution during the crystallization. Figure 4 illustrates the changes in the absorbances at the two characteristic wavelengths during the dissolution and crystallization of caffeine. In addition to quantitative monitoring of solute concentration the absorbance data obtained using the ATR-UV/Vis can be used to detect polymorphic transformation during hydration.

The variation of the absorbance at 274 nm during the solution of caffeine is shown in Figure 6. It can be seen that the absorbance (concentration) shows a peak a few minutes after the anhydrous caffeine was introduced in the water, and the temperature maintained at a constant temperature. After a certain supersaturation is obtained due to the dissolution of the anhydrous caffeine, caffeine hydrate starts to nucleate, which is indicated by the decrease in the concentration. As the temperature increases the caffeine hydrate dissolves and the absorbance starts to increase again, until the clear point is indicated by the significant decrease in the gradient of the signal. The absorbance shows slight dependence on the temperature, which needs to be taken into consideration when the approach is used for quantitative monitoring of the concentration. Figure 6 also indicates that ATR-UV/Vis can be used for automatic detection of the metastable zone for crystallization process. The use of FBRM was also investigated for in situ detection of the polymorphic transformation.

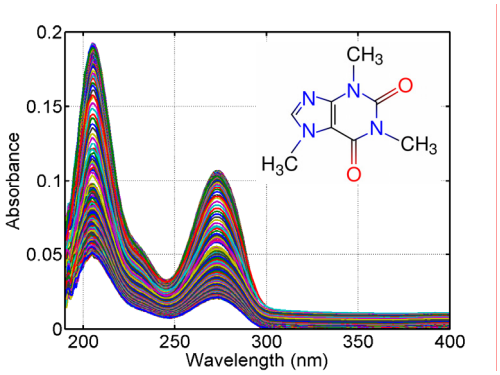

Fig. 4. Variation in absorbance of caffeine during dissolution and crystallization

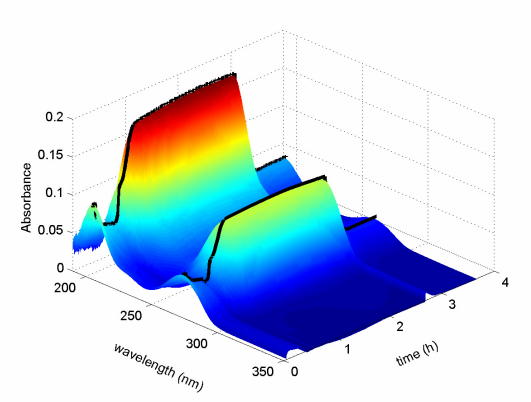

Fig. 5. ATR-UV/Vis absorbance in time showing the dissolution and crystallization of caffeine during the batch when the solution was heated initially and then cooled with constant heating/cooling rates of 0.3 °C/min.

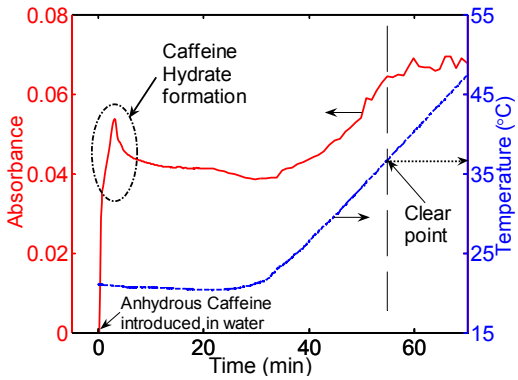

Fig. 6. ATR-UV/Vis spectrum of caffeine showing the transformation of anhydrous form into caffeine hydrate

While the ATR-UV indicates the polymorphic change by detecting the change in the solute concentration due to the incorporation of water into the crystals during hydration, variations in the signal from the FBRM probe convey information related to the change in the shape of the crystals due to the polymorphic transformation. Anhydrous caffeine was introduced in water and changes in the FBRM signal were monitored at constant temperature and agitator speed. Figure 7 shows the variation of the total counts per second and the cube weighted total counts per second. The initial increase in both signals is due the addition of the anhydrous caffeine particles to the water. After addition the signal is approximately constant until polymorphic transformation occurs, after approximately 6 min. In that moment a significant change is detected in both the total count and the cube weighted count. The number of total counts decreases since the small caffeine anhydrate particles dissolve during the formation of long caffeine hydrate needles. Hydration of the caffeine leads to the formation of a voluminous solid phase, which is clearly indicated in the increase in the cube weighted total counts per seconds. The cube weighted statistics is strongly sensitive to change in the large particle sizes, thus it is a good indicator when the polymorphic change involves considerable change in the volumetric shape factor. Although the unweighted total counts per second did show consistently a decrease in the experiments performed the change in the cube weighted counts was more significant in all cases.

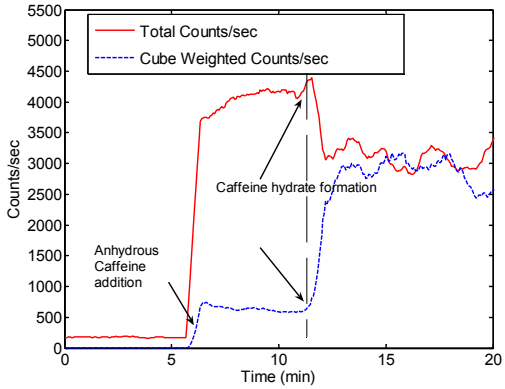

Fig. 7. FBRM signal showing the polymorphic transformation during caffeine hydration.

The FBRM was used to study the effect of agitation and temperature on the kinetics of the hydration. It was observed that as the temperature increases hydration occurs after a longer period of time. Figure 8 shows the effect of mixing on the time interval after which caffeine changes into the hydrate. When the agitator speed was decreased from 200 rpm to 100 rpm the time interval from the addition of anhydrous caffeine to water till the transformation into hydrate increased from 5 min to 12 min.

3.2 Metastable zone determination of caffeine in water using PAT

The main driving force in crystallization is the difference between the chemical potential, which is often expressed in terms of supersaturation, which is the difference between the solute concentration in the actual system and the solubility at the same temperature. Nucleation, growth and polymorphic transformations are driven by changes in the supersaturation. The limits in which the supersturation, hence the driving force of crystallization, can vary are given by the *metastable zone width* (MSZW), which is defined as the difference between the temperature of the clear point (the point when the crystals dissolve) and the cloud points (the point when nucleation of new crystals is detected).

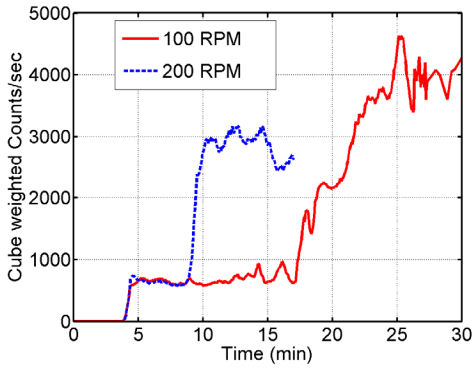

Fig. 8. Effect of agitator speed on the caffeine hydrate formation, monitored by FBRM.

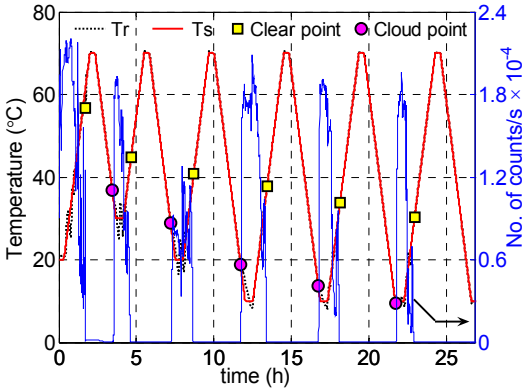

Fig. 9. FBRM and temperature data recorded during the automatic MSZW determination at 200 RPM and 0.5 °C/min (Ts - setpoint and Tr - process temperature).

The MSZW strongly depends on the operating conditions. The effect of different operating conditions (cooling rate, cooling profile, reactor shape, agitator speed and type, etc.) on the metastable zone width (MSZW) and crystal quality were studied and are reported in Gillon et al (2006). The MSZW was determined in each experiment using an automated system. Different cooling/heating rates were used and the clear and cloud points (corresponding to the solubility and nucleation, respectively) were determined using the FBRM, by detecting when the number of counts decreased to the base level (clear point) or started to increase (cloud point). Figure 9 shows sample results from one of the automated metastable zone experiments in the 1L Mettler-Toledo LabMax system, with a 3-blade retreat curve glass agitator, using an initial crystallization volume of approximately 300 mL, which was increased to 800 mL after successive dilutions. Figure 9 illustrates the large MSZW for the primary nucleation of caffeine, which increases as the solution is diluted. The large MSZW is in correlation with the small fine crystals obtained as crystallization occurs from a highly supersaturated solution. The FBRM probe can be used to monitor nucleation and dissolution along the entire MSZW experiment. The variation of the entire chord length distribution along the time is shown on Figure 10. The ATR-UV probe can also be used for automatic MSZW determination.

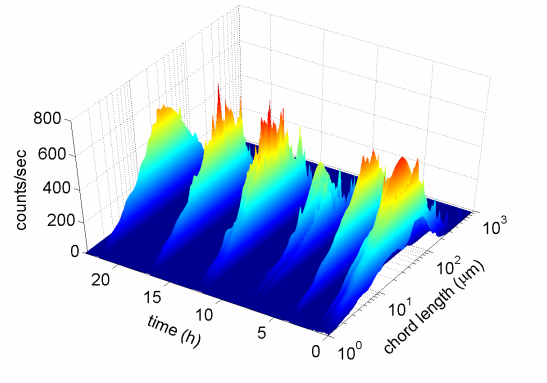

Fig. 10. FBRM and temperature data recorded during the automatic MSZW determination at 200 RPM and 0.5 °C/min.

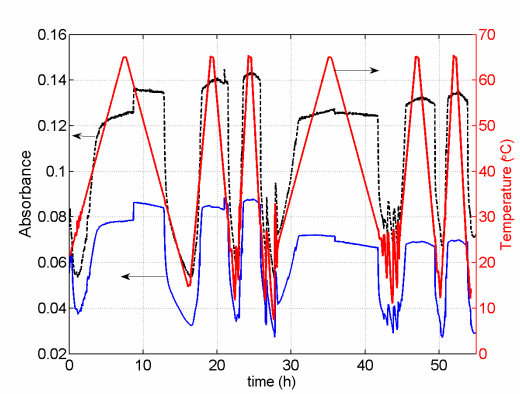

Fig. 11. Automatic metastable zone width determination using ATR-UV/Vis spectroscopy.

Figure 11 illustrates the variations in the absorbance at the two characteristic wavelength during the metastable zone experiments at two different concentrations and three cooling/heating rates (0.1, 0.3 and 0.5 °C/min).

Data obtained from the metastable zone experiments can be used for estimation of the nucleation kinetics. Consider that the nucleation rate is given by the power law, $B = k_b (C - C^*)^b$, where B is the nucleation rate, C is the solute concentration, C^* is the solubility equation, and k_b and b are parameters for the nucleation kinetics. According to the method proposed by Nyvlt, if a solution with a fixed initial concentration is cooled down with different but constant cooling rates (β) there is a linear dependence between the metastable zone width (MSZW) and the cooling rate, given by:

$$\ln(\beta) = b \ln(MSZW) + K \qquad (1)$$

with $K = (b-1)\ln(dC^*/dt) + \log k_b$, with t being the time. Figure 12 shows the results corresponding to the MSZW experiments shown on Figure 11. The average nucleation exponent obtained at the two different concentration is $b = 3.18$ (3.10 and 3.26 respectively). Note that the value obtained for b is the apparent nucleation kinetics, which assumes spontaneous nucleation with initial size for the nuclei corresponding to the size when the nucleation is detected.

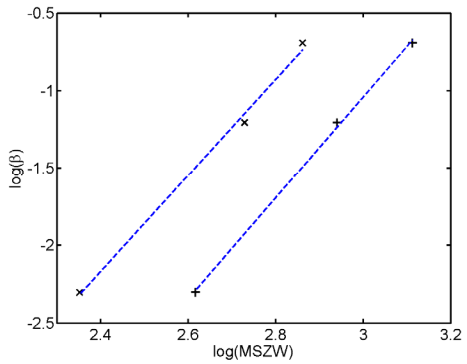

Fig. 12. Linear dependence between cooling rate and MSZW according to Nyvlt.

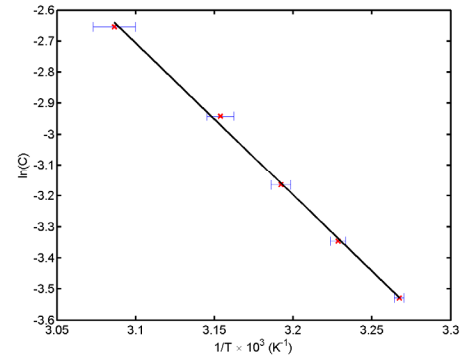

Fig. 13. Van't Hoff solubility curve for caffeine in water.

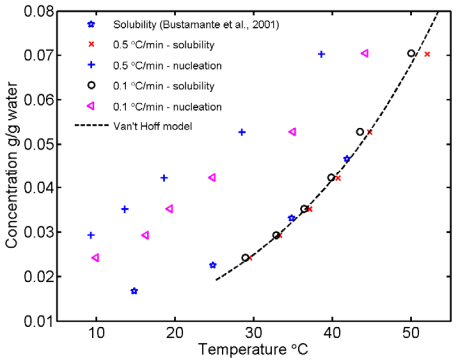

Fig. 14. Solubility and metastable zone width of caffeine in water at different cooling rates.

The solubility model of caffeine in water was obtained using the Van't Hoff equation,

$$\ln(C) = -\frac{\Delta H_d}{R}\frac{1}{T} + \frac{\Delta S_d}{R}, \qquad (2)$$

where C is the mass fraction of solute (g caffeine/g water), ΔH_d and ΔS_d are the enthalpy and entropy of dissolution, respectively, R the gas constant (8.31 $\text{Jmol}^{-1}\text{K}^{-1}$) and T is the saturation temperature. Figure 13 shows the linear plot between $\ln(C)$ and $1/T$. The slope and intercept of the linear plot yields $\Delta H_d = 40.8$ kJmol^{-1} and $\Delta S_d = 104.0$ $\text{Jmol}^{-1}\text{K}^{-1}$. Figure 14 shows the solubility curve obtained with the automated approach, and the model given by (2). The solubility data obtained is in good correlation with the values reported in the literature (Bustamante et al, 2002), with a low sensitivity on variations in the cooling rate. Figure 13 also illustrates the increase in the MSZW as the cooling rate increases, which is in good correlation with experimental observations of generic crystallization processes.

4. CONCLUSIONS

The use of different process analytical technologies (PAT) for the *in situ* detection of the polymorphic transformation of anhydrous caffeine into hydrate is exemplified. Different PAT, such as FBRM, ATR-UV, and PVM were successfully used for the monitoring of the polymorphic change during the transformation of anhydrous caffeine into hydrate and aqueous solutions. It was shown that increase in agitation and decrease in temperature enhances the formation of caffeine hydrates. Application of PAT for the thermodynamic and kinetic study of caffeine crystallization is also exemplified. Automatic metastable zone width experiements are used to determine the dissolution enthalpy and entropy, as well as the apparent nucleation kinetics for the model pharmaceutical compound.

ACKNOWLEDGEMENTS

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