Combined PAT-Solid State Analytical Approach for the Detection and Study of Sodium Benzoate Hydrate

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Abstract:

This paper presents the application of process analytical technology (PAT) tools in combination with solid state analytical (SSA) techniques for the detection of solvent-mediated pseudopolymorphic transformations and the discovery of a new hydrate form of the model system, sodium benzoate in water. Cooling crystallization experiments were performed at a range of scales including 1 mL, 250 mL, and 1 L, using turbidity and focused beam reflectance measurement (FBRM) probes. The solubility determination using the turbidity probe indicated a very low solubility of the sodium benzoate crystals in organic solvents but high solubility in water, with low temperature sensitivity in all cases, making the control of cooling crystallization challenging. Monitoring the cooling crystallization of sodium benzoate from water using focused beam reflectance measurement (FBRM) probe indicated the formation of sodium benzoate hydrate, which has not been reported before. Additionally, a combination of off-line solid state techniques were used, including optical and scanning electron microscope (SEM), hot stage microscopy (HSM), X-ray powder diffraction (XRPD), nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and dynamic vapour sorption (DVS), to confirm the formation of the new channel hydrate, when sodium benzoate recrystallizes from water.

1. Introduction

Crystallization is a key unit operation in the manufacturing of pharmaceutical products. It is estimated that about 80% of all pharmaceutical products require a crystallization step.[1] A common approach for the design of this step involves trial and error methods in which an undersaturated solution is cooled down at a constant ramp rate, resulting in a supersaturated solution. The degree of supersaturation is the driving force for crystallization.[2] Despite the long history of using crystallization as a unit operation, the process often leads to failed batches due to the crystallization of different polymorphic forms, which may result in an increase in economical costs.[3] Additionally, most pharmaceutical compounds are able to incorporate solvent molecules during crystallization, leading to the formation of

different crystal structures, sometimes referred to as pseudopolymorphs (hydrates or solvates).[4] Different polymorphic or pseudopolymorphic forms have the same chemical composition but have different arrangements of molecules, resulting in different structures. This leads to variations in morphology and physicochemical properties between the different forms, including melting point, stability, solubility, dissolution rates, and bioavailability, which can have a great effect on the quality of the product and can lead to problems with downstream processing.[5] The most well-known example is the case of the pharmaceutical Ritonavir. Abbott Laboratories discovered a new polymorphic form of the drug Ritonavir after it had been on the market for two years.[6] Differences in the solubility of the two forms resulted in failed dissolution tests in the originally semisolid capsule which lead to a market crisis.[6] There are three different classifications of hydrates including channel, isolated site, and ion-associated hydrates. Channel hydrates contain channels of loosely bonded water molecules throughout the crystal lattice structure.[7] These hydrates are also known as nonstoichiometric hydrates in which the ratio of water to drug molecule is less than 1.[8] It is of very high importance within the pharmaceutical industry that all possible polymorphs, hydrates, or solvates of a particular compound are identified and the crystallization process is controlled so that the desired form is produced.[9]

Recent advances in the pharmaceutical industry have involved the use of process analytical technology (PAT), which has been greatly promoted by the Food and Drug Administration (FDA) regulatory body.[10] These tools enable the systematic design and control of the crystallization systems to produce high-quality crystalline products with reduced variability in the properties.[11−14] PAT tools are also used for the *in situ* monitoring and detection of different polymorphic/pseudopolymorphic

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forms. Focused beam reflectance measurement (FBRM) probe is a widely used PAT tool that measures the changes in chord length distribution of a crystal population. This tool has been used for the detection of polymorphic transformations that result in a change in crystal size or morphology.[15−21]

Process analytical tools can be used to indicate a potential polymorphic transformation; however, a range of solid state analytical (SSA) techniques usually must also be used to confirm the formation of polymorphs. These techniques provide information that enables the different polymorphic/pseudopolymorphic forms to be identified. Various combinations of the SSA techniques have been used for the identification of polymorphic forms, including X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), dynamic vapour sorption (DVS), and a range of microscopy techniques including hot stage (HSM), and optical and scanning electron microscope (SEM).[7,22−25]

This paper presents the advantages of using PAT tools combined with SSA techniques for the monitoring and detection of different polymorphic/pseudopolymorphic forms of a model system, sodium benzoate. As there is limited literature data on the solubility of sodium benzoate, the first stage of experiments involved solubility screening using the Avantium Crystal 16 unit on a 1 mL scale. Scale-up experiments were performed from the 1 mL to vessel sizes of 250 mL and 1 L for the cooling crystallization of sodium benzoate from water. The main PAT tool used within these studies was the FBRM probe, which detected the formation of an unknown hydrate. A series of solid state analyses were carried out on the crystals using a range of techniques including DSC, TGA, DVS, XRPD, HSM, optical microscopy, SEM, solid state NMR and IR spectroscopy. The results provide evidence of the existence of a new channel hydrate of sodium benzoate.

2. Experimental Materials and Equipment

2.1. Materials. Sodium benzoate was used as the model system for all experiments. The raw material (sodium benzoate) is the anhydrous form and was obtained from two different sources. These included Sigma Aldrich and Fisher Scientific, both which had of purity greater than 99.0%. Solubility studies were carried out using a range of solvents including water, methanol, ethanol, propan-2-ol, butan-2-ol, acetone, acetonitrile, methyl ethyl ketone (MEK), and tetrahydrofuran. Ultrapure Millipore water generated from a Milli-Q reverse osmosis unit was used, and all other solvents were of analytical reagent grade, which were obtained from Fisher Scientific.

2.2. Solubility Measurements. Solubility screening experiments were performed at 1 mL scale using the Avantium Crystal 16 which contains reactor blocks of 4 by 4 of 1 mL HPLC vials agitated by magnetic fleas which were set at a rotation speed of 700 rpm. Each vial contained a turbidity sensor which was used to detect the clear (all crystals dissolved) and cloud points (apparent nucleation). The temperature was controlled by a Huber ministat which was connected to a PC. Three experiments were set up using water, alcohols (methanol, ethanol, propan-2-ol, and butan-2-ol) and four other organic solvents (acetone, acetonitrile, MEK, and tetrahydrofuran). For all experiments a ramp rate of 0.3 °C/min was used. Solubility data for sodium benzoate in water was obtained using a gravimetric method. Suspended solid solutions were shaken in the water bath for 24 h, and samples of 0.5−1 mL were then placed in an oven at 25 °C for 22−24 h for solvent evaporation.

2.3. Cooling Crystallization Experiments at Scales of 250 mL and 1 L. Cooling crystallization experiments were performed with the use of the FBRM probe at the 250 mL and 1 L scale, using the model system sodium benzoate in water. FBRM probe was used to detect the change in number of counts with chord lengths within a size ranges of 1−20 μm (fine counts), 50−250 μm (coarse counts), and 1−1000 μm (total counts). A 90 logarithmic channel mode was used to collect data into specific bins. This PAT tool can be used to detect nucleation, growth, agglomeration, attrition, and polymorphic transformations. The 250 mL scale experiments were carried out using a three-blade glass retreat curve agitator and using a D600l-HC-K FRBM Lasentec probe. The 1 L scale experiments were conducted using a stainless steel three-blade marine agitator and an A100 Lasentec FBRM probe. In all experiments a stirrer speed of 300 rpm was used, whereas the ramp rates and initial concentrations varied. Ramp rates of 0.1, 0.3, and 0.5 °C/min were used at initial concentrations of 466, 566, and 633 mg/mL of solvent. The temperature was controlled using a Huber Ministat unit. For the 250 mL scale this was connected to a computer package known as Labworldsoft which controlled the temperature at a desired set point. For the 1 L scale experiments both FBRM and temperature are recorded using a software tool developed in Labview. Figure 1 shows both the schematic of the setup and picture of the crystallization rig.

2.4. Solid State Analysis of Crystals. Solid state analyses of the crystals were carried out using an Olympus BX51 microscope with a digital camera and image Pro Plus software. HSM consisted of the same microscope with a Linkam heating stage attachment connected to a 3-CCD colour video camera (JVC model KY-F55B). This technique and a Perkin-Elmer DSC (Pyris 1) provided data on the melting points of the polymorphs. A Perkin-Elmer (Pyris 1) TGA and a DVS unit were used for the analysis of the hydrates. For both DSC and TGA all samples were analysed using a heating rate of 10 °C/min. XRPD was carried out on crystal samples, which required gently crushing the samples into a fine powder to prevent

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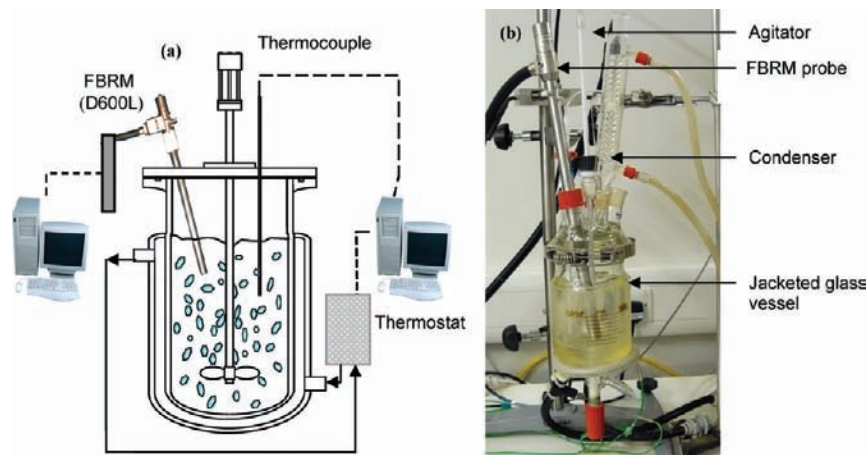(25) Giron, D. *Thermochim. Acta* **1995**, *248*, 1–59.

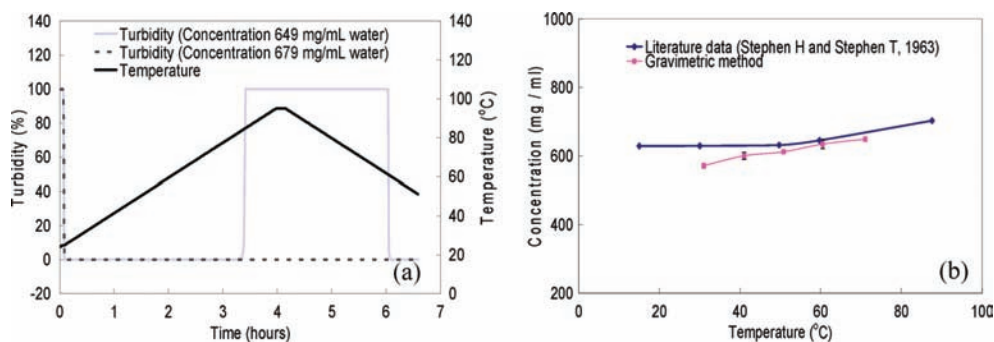**Figure 1.** (a) Schematic of the setup and (b) picture of the crystallization rig.

Figure 2. Solubility results for sodium benzoate in water obtained from (a) Crystal 16 unit (turbidity points) and (b) literature data[22] and gravimetric method.

preferred orientation. A scan speed of 1°/min was used, and the start and stop angles were 3° and 35°, respectively. Solid state NMR data were obtained by samples placed within 4 mm OD rotors and spun at 12 kHz using a Bruker Advance III NMR spectrometer. IR data were obtained using a Perkin-Elmer instrument (FT-IR system) of model spectrum GX.

3. Results and Discusion

3.1. Solubility Measurements. The main aims of the experiments were to use PAT tools combined with solid state techniques to identify the different polymorphic forms of sodium benzoate. The first stage of the experiments involved solubility screening in order to find a suitable solvent for which sodium benzoate can be recrystallized using cooling. For the screening experiments Avantium Crystal 16 equipment was used. This detects the cloud and clear points using turbidity probes. A turbidity of 100 represents a clear solution which indicates that all the crystals have dissolved, and a turbidity value of zero indicates a cloudy solution with crystals present. Turbidity values for organic solvents remained at 0% for the concentration range 20−40 mg/mL, indicating that sodium benzoate has a very low solubility in organic solvents. Figure 2a shows the turbidity results for the cooling crystallization experiments using water as a solvent. A concentration of sodium benzoate in water of 649.8 mg/mL results in a clear point of 85 °C indicating that sodium benzoate has a high solubility in water. The solubility obtained using gravimetric analysis is slightly lower when compared with literature data, as shown in Figure 2b; however, both these results show a similar solubility curve over

the temperature range 30−70 °C. Literature solubility data show that for the operating temperature range of water between 15 and 97 °C the solubility of sodium benzoate is between 628 and 733 mg/mL, indicating a high solubility but small temperature sensitivity of the solubility (flat solubility curve). In the interval 10−50 °C, there is practically no change in solubility, and even in the range of 50−90 °C, the change is only about 10%. Due to the high solubility, water was chosen as the solvent for the cooling crystallization experiments, and initial concentrations and operating temperature were carefully selected to be able to crystallize the compound via cooling.

3.2. Monitoring of Sodium Benzoate Hydrate Formation Using PAT Tools. Experiments were performed at 250 mL and 1 L scales with the use of the FBRM probe to monitor the cooling crystallization of sodium benzoate from water. Figure 3 shows the FBRM results for the 1 L and three repeated experiments using a 250 mL vessel with variations in the initial concentrations of sodium benzoate in water of 466, 566, and 633 mg/mL solvent. The agitator types were varied within the differently sized vessels to examine the effect of mixing conditions on the behaviour of the system. In all cases, as the system was heated, the crystals dissolved as indicated by a decrease in the FBRM counts. The larger baseline in the case of the 1 L vessel (Figure 3a) is due to the use of an older FBRM probe (model A100) with small defects on the window. At temperatures above 65 °C there is a fluctuation in the number of counts which is thought to be due to a polymorphic transformation as it is repeatable within the same vessel using different concentrations. For a concentration of 566 mg/mL,
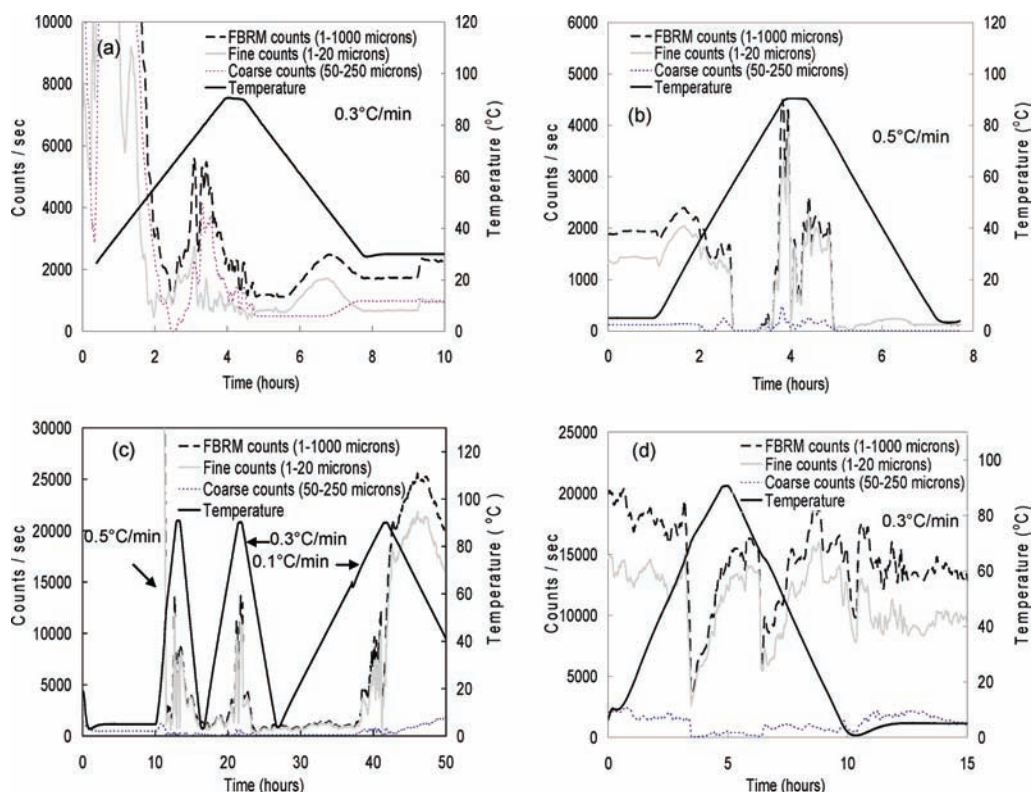
Figure 3. FBRM results for the cooling crystallization of sodium benzoate in water at different initial concentrations (C_0): (a) 1 L vessel and C_0 = 466 mg/mL; (b) 250 mL vessel and C_0 = 466 mg/mL; (c) 250 mL vessel and C_0 = 566 mg/mL; (d) 250 mL vessel and C_0 = 633 mg/mL.

the fluctuations occurred at all ramp rates of 0.1, 0.3, and 0.5 °C/min. The repeatability of the results using different mixing conditions, within the different vessel sizes, indicates that this factor does not affect the behaviour of the system. The use of a combination of FBRM statistics including fine (1−20 μm), coarse (50−250 μm), and total (1−1000 μm) counts shows that the FBRM signal is not due to attrition or agglomeration as all counts show a similar behaviour during the fluctuation, providing further evidence of a possible polymorphic transformation. Similar results for other model systems are reported in the literature, which involved the use of FBRM statistics to indicate a polymorphic transformation from a metastable to a stable form.[15,18,27] However, it has to be mentioned that the FBRM signal is very difficult to interpret since various events during the crystallization may lead to similar changes in the FBRM signal.[28] Nevertheless, FBRM can provide a very useful tool to signal that an event happens in a particular stage of the crystallization process, which generally requires additional solid-state analytical methods to elucidate. As hydrates tend to be stable at lower temperatures and anhydrates tend to stable at higher temperatures,[9] it is predicted that there is a polymorphic transformation from a hydrate to the anhydrate form of sodium benzoate at a temperature above 65 °C. This transformation occurs without complete dissolution of the anhydrous form (FBRM counts do not decrease to zero as shown in Figure 3d).

**3.3. Evaluation of the Formation of the New Sodium Benzoate Hydrate Using Solid State Analytical Methods.** To investigate whether a hydrate forms when sodium benzoate recrystallizes from water, samples were taken from the cooling crystallization experiments and analysed using a range of SSA techniques, such as optical and SEM, HSM, XRPD, DSC, TGA, DVS, and NMR and IR spectroscopy.

Figure 4 shows the optical and SEM images of the anhydrous form of sodium benzoate and samples recrystallized from water. The optical microscope image of the anhydrous form shows large agglomerates made up of many fine particles of sizes less than 10 μm. The SEM image shows the particles have a rough surface. SEM images of the sample recrystallized from water show the formation of a solid clump which has a fibrous surface structure. This fits with the optical microscope images which show long fibrous needles with longest dimension of approximately 200 μm. Any differences in these images is due to the fact that the optical microscope image was obtained from a mobile slurry and the SEM images were obtained after the crystals had been filtered under vacuum, resulting in the formation of the solid clump. Comparing the SEMs of the raw material (anhydrous) and sodium benzoate recrystallized from water, shows a clear difference between the surface characteristics. The change in morphology from fines to needles indicates the possibility of a different form (hydrate). Nagy et al.[18] reported similar results for the detection of a polymorphic transformation of caffeine from anhydrous form to a channel hydrate that resulted in a change in morphology from fines to

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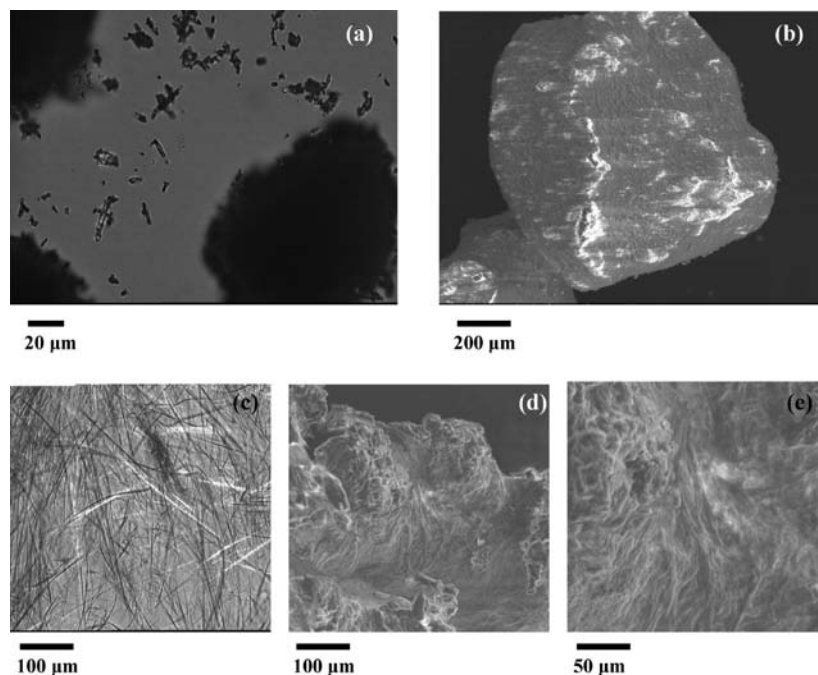
Figure 4. (a) Optical microscope image of raw material (anhydrous) form of sodium benzoate, (b) SEM image of raw material (anhydrous) form of sodium benzoate, (c) optical microscope image of sodium benzoate recrystallized from water, and (d, e) SEM images of sodium benzoate recrystallized from water.
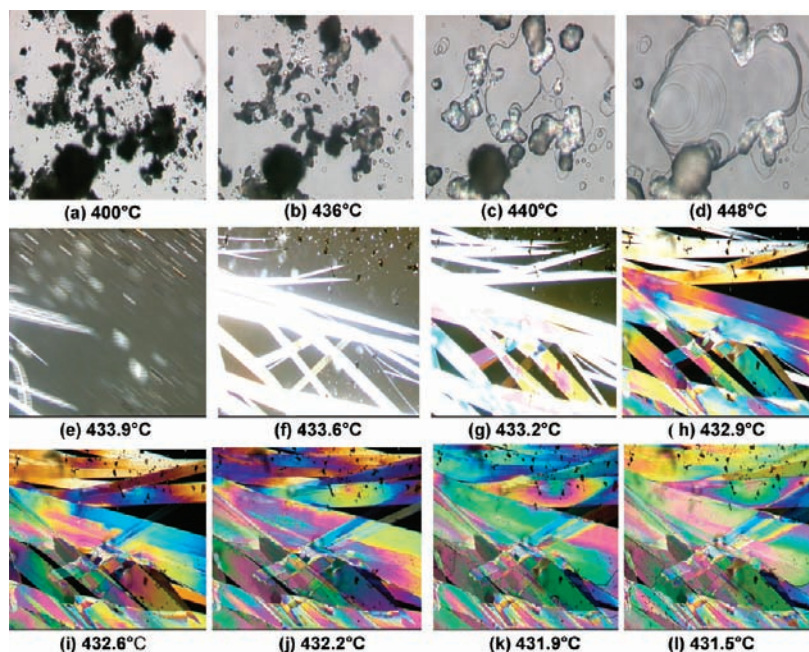
Figure 5. Hot stage microscopy (HSM) images of sodium benzoate. Images (a–d) are during the heating phase and (e–l) are during the cooling of the melt.

needles. During the cooling crystallization of sodium benzoate from water a solid clump formed which is immobile due to the result that sodium benzoate uses up water during the formation of the hydrate.

Figure 5 shows the HSM results for the anhydrous form of sodium benzoate. The HSM images show that the crystals start to melt between 436 and 440 °C which is considered very high for organic compounds even if sodium benzoate is an organic ionic compound (salt). HSM images also show that sodium benzoate recrystallizes from the melt showing a birefringent crystal behaviour

with an anisotropic structure, also illustrated in Figure 6. These compounds are known to exhibit a tendency to form polymorphs. HSM was performed on the sample of sodium benzoate recrystallized from water (hydrate). Brittian[7] reported hot stage microscopy as a useful technique in classifying the type of hydrate. For channel hydrates, dehydration is observed as a progressive darkening of the ends of the crystal towards the centre as the crystals are heated. However, these results showed no change in the appearance of the crystals during the temperature range 25−440 °C (melting point of anhydrous form). This could

Figure 6. Birefringent crystal of the anhydrous sodium benzoate crystal recrystallized from melt.

be due to the fact that the crystals appeared as dark, solid agglomerates made up of very fine fibres as shown by the SEM images in Figure 4d,e.

Further analysis was carried out using XRPD. This technique is used for the identification of different polymorphic forms of a given compound as each polymorph is represented by a particular X-ray diffraction pattern.[29,30] Figure 7 shows the XRPD patterns for the raw material, the anhydrous form of sodium benzoate. and the sodium benzoate recrystallized from water. The anhydrous sodium benzoate for these tests was produced by heating the crystals obtained from the crystallization in water above the dehydration temperature, using TGA. These XRPD results show similar patterns for the raw material and the anhydrous form, indicating that the raw material is anhydrous; however, the pattern for sodium benzoate recrystallized from water shows additional and missing peaks, indicating a different internal structure compared to the anhydrate form. This provides evidence that a different polymorphic form exists.

NMR spectroscopy is a powerful technique for identifying different forms of a compound including (nonstoichiometric and stoichiometric) hydrates and anhydrates.[31,32] This technique is used with XRPD to provide supporting evidence on the presence of different forms of a compound as related to geometry and compositional differences. The different chemical environments exposed to the carbon and hydrogen atoms within the molecule are represented by specific peaks in the NMR spectrum. Figure 8 shows the solid state NMR data of ^{13}C, ^{1}H, and ^{23}Na for the anhydrous form of sodium benzoate and the form recrystallized from water. ^{13}C data for both forms is very similar, showing four peaks representing four different chemical environments. The peak at 177 ppm is due to the carboxylate carbon (COO^-), and the three peaks within the range 120−140 ppm are due to the carbon atoms in the aromatic ring of sodium benzoate.[33] ^{23}Na spectra show a broad peak for the anhydrous form of sodium benzoate representing immobile sodium ions, while the spectra for the sample recrystallized from water show an additional sharp peak at 1 ppm. The sharp peak represents

mobile sodium ions providing supporting evidence of the presence of water molecules within the internal structure of the crystal. ^{1}H data for the anhydrous form show a broad peak in the range 0−10 ppm, while the spectra for the sample recrystallized from water shows two sharp peaks in the range 4−6 ppm which has been reported to be due to the −OH group of a molecule indicating the presence of a hydrate.[34]

An additional spectroscopy technique involving infrared (IR) is applied to the anhydrous and sample recrystallized from water of sodium benzoate. The IR data is shown in Figure 9. There are two additional peaks in the IR spectra at wavenumbers of 3440 and 3600 cm^{-1} for the sample of sodium benzoate recrystallized from water. IR data on a hydrated form of a compound showed peaks at wavenumbers within the region of 3400−3700 cm^{-1} and a sharp peak at 3600 cm^{-1} which is due to the hydroxyl group (−OH) of water molecules.[35] This data provide supporting evidence that the peaks at wavenumbers 3440 and 3600 cm^{-1} in the IR spectra for sodium benzoate recrystallized from water are due to the presence of water molecules within the crystal lattice structure, signifying the formation of a hydrate.

Further solid state analysis was performed using DSC. Figure 10 shows the DSC results for the raw material (anhydrate) form of sodium benzoate and sodium benzoate recrystallized from water. The type of pan used is a sealed pan with a pinhole to prevent the build up of pressure. DSC results for the raw material (anhydrous) show a sharp peak at 440 °C which confirms the results obtained from HSM, of a high melting point for the organic compound sodium benzoate. The enthalpy of melting for the raw material is 164 J/g. Figure 10b shows the DSC results for the sample of sodium benzoate recrystallized from water, showing two peaks. The reason for the slight differences in the peak positions between the two repeated experiments is due to the analysis being carried out using a different DSC machine and sample material. The first peak occurs within the temperature range 75−118 °C, which is below the melting point of the anhydrous form. The sample under analysis has not been completely air-dried, indicating that this is due to water on the surface of the crystals. The second peak within the temperature range 95−125 °C could be due to the dehydration of a hydrate. Giron[25] reported DSC results for a hydrated form of a compound which showed the dehydration process as a single endothermic peak occurring within the temperature range 70−130 °C. This also suggests that the second peak within the temperature range 95−125 °C in the DSC results for sodium benzoate recrystallized from water is possible due to the dehydration of a hydrate. The enthalpy of dehydration is 10 J/g. The low enthalpy change signifies the formation of a channel hydrate as not much energy is required for removal of water from the crystal lattice structure due to the loosely bonded water molecules throughout the crystal lattice structure. For channel hydrates, once dehydration has occurred, this forms a crystal structure of low density with empty voids, resulting in an unstable structure.[7] The crystal lattice parameters may then alter to obtain a stable structure. From DSC analysis

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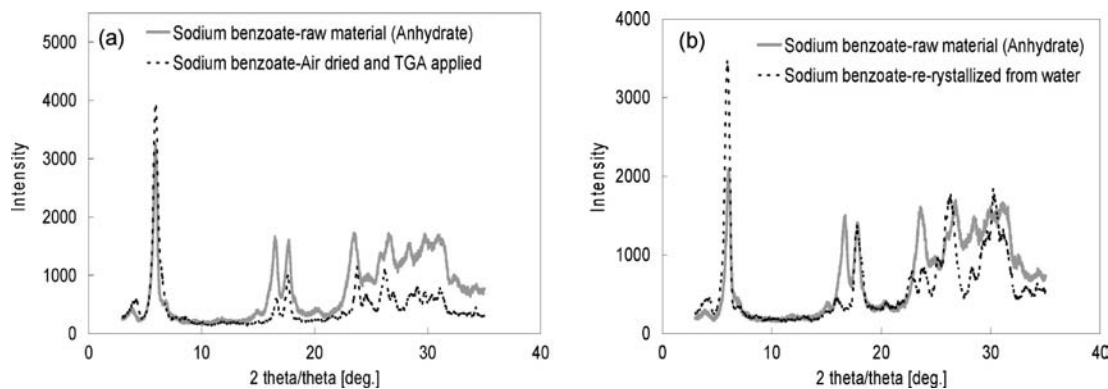(31) Harris, R. K.; Jackson, P. *J. Phys. Chem. Solids* **1987**, *48*, 813–818.
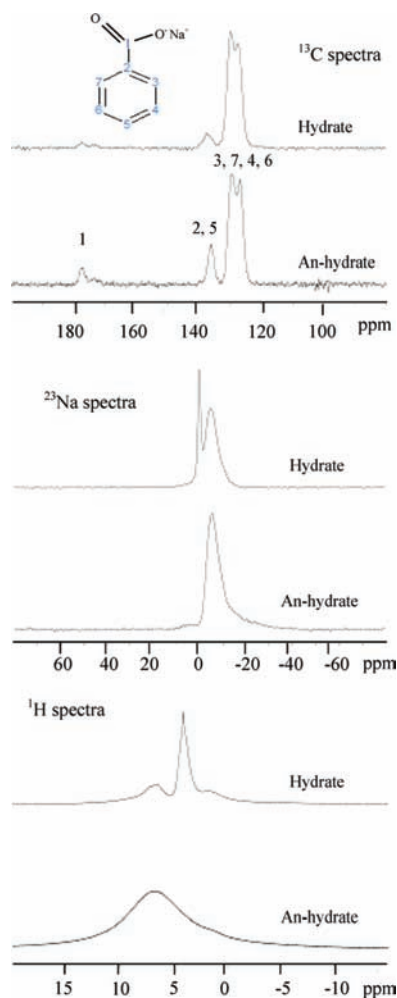(32) Harris, R. K. *J. Pharm. Pharmacol.* **2007**, *59*, 225–239.
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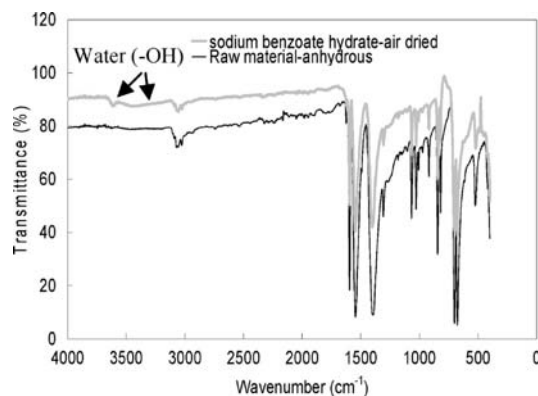
Figure 7. XRPD results for (a) raw material and sodium benzoate (air-dried and TGA applied) and (b) raw material and sodium benzoate recrystallized from water.

Figure 8. NMR data of ¹³C, ²³Na and ¹H spectra for the anhydrate and hydrate form of sodium benzoate.
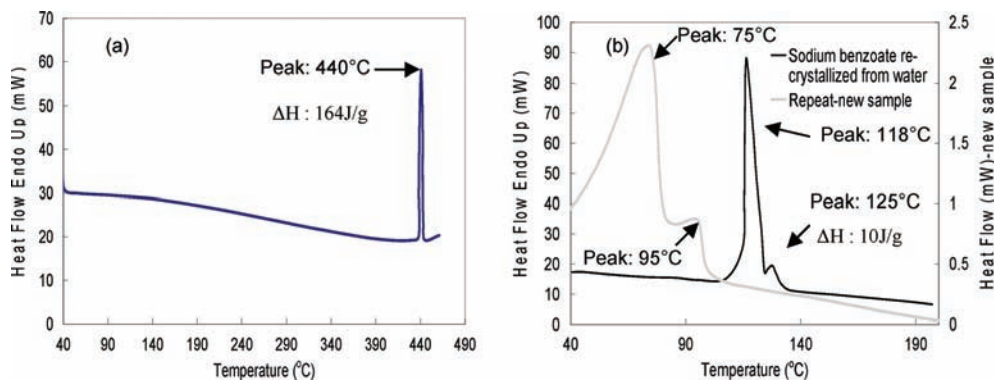

**Figure 9.** IR spectra for the anhydrous form and recrystallized from a water sample of sodium benzoate.

stoichiometry of sodium benzoate to water is calculated. The mean molecular ratio of sodium benzoate to water is 1:0.28 with standard deviation of ±0.02. The nonstoichiometric ratio and the relatively low temperature at which the dehydration occurred indicate that the hydrate formed is a channel hydrate.

DVS studies are very useful for classifying hydrates and to determine whether the material is amorphous or highly crystalline. Amorphous or deliquescent materials absorb large amounts of water at high relative humidity, whereas highly ordered crystalline materials can absorb very small amounts of water of up to 0.1% weight increase.[8] Stoichiometric hydrates show sudden increases in water uptake, whereas nonstoichiometric hydrates (channel hydrates) show a small and gradual increase in water uptake as the relative humidity is increased from 0 to 90%.[8] DVS was applied to a sample of anhydrous sodium benzoate to see how this behaves when subjected to different amounts of water. This is achieved by changing the relative humidity (RH) between 0 and 90% while keeping the temperature constant at a value of 24.7 °C. As the RH increases, any uptake in water results in an increase in mass, and the reverse is true when the RH is lowered. The results for two repeated experiments are shown in Figure 12.

DVS results show a large increase in the mass % as the RH is increased from 80 to 90% which suggests that during this phase sodium benzoate deliquesces.[8] For cycle 2 during the sorption stage as the relative humidity increases from 40% to 60% there is a small and gradual increase in the mass of sodium benzoate. Both desorption cycles also show a small but significant decrease in mass % as the RH is decreased from 30

this confirms that the anhydrous form is stable above the dehydration temperature of 125 °C.

To support the prediction that a channel hydrate forms, TGA analysis is performed on the sodium benzoate crystals. Figure 11 shows the TGA results for the air-dried sodium benzoate crystals obtained at the 1 and 250 mL scales. Similar results are obtained at both scales showing a wt % decrease between 3 and 3.7% over a temperature range of 60−80 °C. The decrease in mass represents a loss in water from the internal structure of the crystals. On the basis of the wt % decrease, the

Figure 10. DSC results for the (a) raw material form of sodium benzoate and (b) samples of sodium benzoate recrystallized from water.
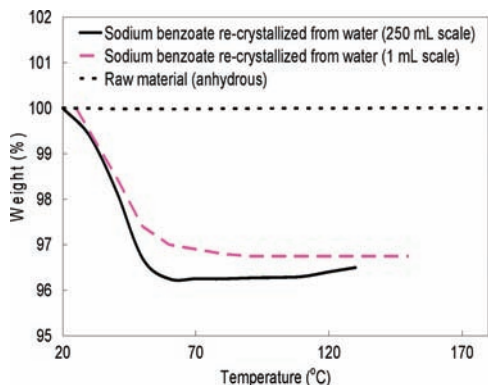
Figure 11. TGA results for the raw material form of sodium benzoate and sodium benzoate recrystallized from water at the 1 and 250 mL scale.
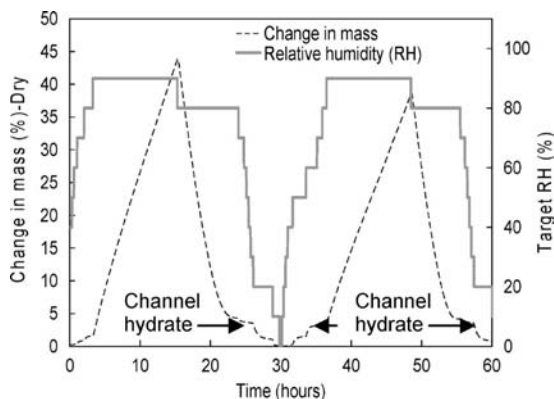
Figure 12. DVS results for sodium benzoate showing how the sample mass and relative humidity change with time.

to 10%. This change is significant enough to suggest the formation of a hydrate. The type of hydrate is assumed to be a nonstoichiometric (channel) hydrate as the wt % change is small and gradual, which is a common behaviour for channel hydrates.[8] These observations reinforce the results obtained from TGA which also indicated the formation of a channel hydrate when sodium benzoate recrystallizes from water. From the small and gradual change in mass during each step in the DVS results the molecular ratio of sodium benzoate to water is calculated, and a summary of the results are shown in Table 1. The results show a mean molecular ratio of sodium benzoate to water of 1:0.27 with standard deviation of ±0.02. These results are very similar to those obtained from TGA analysis which gave a mean value of 1:0.28 and standard deviation of ±0.02. Studies carried

Table 1. Summary of the DVS results for the molecular ratio of sodium benzoate to water

step	change in humidity (%)	change in mass (%)	molecular ratio of sodium benzoate to water
sorption (cycle 2)	40−60	2.89	1:0.24
desorption (cycle 1)	30−10	3.47	1:0.29
desorption (cycle 2)	30−10	3.53	1:0.29

out on the hydration of caffeine showed similar findings involving the formation of a nonstoichiometric hydrate with a molecular ratio of caffeine to water of 1:0.8.[36] The above results from both TGA and DVS confirm that a hydrate forms from the recrystallization of sodium benzoate from water, which has a molecular ratio of sodium benzoate to water of 1:0.28, leading to the conclusion that a channel hydrate forms which has not been reported before.

4. Conclusions

Cooling crystallization experiments were performed with the use of the FBRM probe which detected an unexpected fluctuation in the total number of counts above 65 °C. Repeated cooling crystallization experiments under different conditions showed very similar behaviour. The fluctuation indicated that probably a polymorphic transformation occurred during that stage of the crystallization process. The use of PAT combined with SSA techniques provided evidence of the formation of a new hydrate when sodium benzoate recrystallizes from water. TGA and DVS confirmed that the molecular ratio of sodium benzoate to water in the new hydrate is 1:0.28 indicating the existence of a previously not reported channel hydrate.

(36) Pirttimaki, J.; Laine, E. *Eur. J. Pharm. Sci.* **1994**, *1*, 203–208.