CrystEngComm

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/crystengcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

In-situ Raman mapping for identifying transient solid forms.

Jagadeesh Babu Nanubolu[a] and Jonathan C Burley[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

Paracetamol trihydrate is a highly unstable form which undergoes a rapid phase transformation to its stable anhydrous polymorph I. In-situ Raman surface mapping on trihydrate sample provides an evidence for a transient phase on the route to its stable polymorph I. The transient phase showed significant spectral differences from those of known polymorphs, trihydrate and amorphous form, and is tentatively assigned to the monohydrate on the basis of observed crystallite morphology.

Introduction

Polymorphs are different crystalline forms of the same drug without a change in its molecular composition.[1] Understanding the polymorphism (the ability of the drug to crystallize as multiple crystalline forms) and the solid form stability are important research activities in pharmaceutical industries owing to their considerable differences in physicochemical properties and as patentable inventions.[2] The metastable solid forms provide solubility advantages over the stable ones, however, they pose several stability challenges, such as unwanted phase transformation, chemical degradation, partial/or full dehydration and etc, which need to be investigated and addressed in the drug formulation stage.[3]

Over the years, Raman spectroscopy has evolved as an important analytical tool for molecular identification and solid form characterization (polymorphs, cocrystals, salts, amorphous, solvates and etc).[4] The use of high sensitive detectors for detecting the feeble Raman scattering signals, confocal set up for non-contact operation, improved optics, and fast data collection times in milliseconds facilitate the surface mapping of materials[5] almost in real time and offer important spatial insights on the dissolution mechanism,[6a] crystallization,[6b] phase transformations,[6c,6d] solid dispersions,[6e] co-crystal formation,[6f] phase purity[6g] and detection of low-content API formulations.[6h] A typical Raman mapping[5] involves sequential measurements on adjacent regions of the sample by point-by-point scanning. Each point of the sample is moved into the beam focus of the microscope for spectral data collection. Raman spectra collected in mapping experiments are processed by statistical routines to generate meaningful plots which provide the chemical composition and spatial distribution. Raman chemical imaging,[7a,$] a slightly different method compared to Raman surface mapping,[5] is routinely employed in biomedical applications for understanding the cell composition, cell division and for disease diagnosis.[7]

Realizing the advantages offered by Raman spectroscopy for solid form identification, we employed surface mapping in one of our previous studies to address a long standing issue of peculiar recrystallization behavior of paracetamol,[6b] an analgesic drug used for the treatment of fever. Paracetamol exists in three well characterized anhydrous polymorphs (I, II, III) and an amorphous form.[8] Interestingly the amorphous material recrystallizes to different polymorphs under slightly different experimental conditions which puzzled researchers for several years.[9] We employed covered and uncovered conditions to study this interesting recrystallization behaviour.[6b] The amorphous material recrystallized to metastable form III in covered conditions, whereas it directly recrystallized as form I/II in uncovered conditions. The time interval Raman surface maps provided a crucial missing link that related the role of surface versus bulk nucleation[10] to different recrystallization pathways. In the uncovered material, the crystallization occurred at the surface and gradually extended to bulk material, whereas in the covered material, the crystallization at the surface was inhibited favoring only bulk material to reorganize. As a result, the crystallization rates and pathways were severely affected. Uncovered material crystallized in less than an hour while covered material required more than six weeks to complete crystallization. Other factors like, subtle differences in the amorphous states and their possible role on the recrystallization were addressed using the Raman spectra.[6b]

As an extension of our work on this model system paracetamol, we intend to employ Raman mapping on rapidly converting metastable forms to understand the phase transformation events. Two hydrated forms of paracetamol were reported under ambient conditions; a trihydrate form[11a,b] crystallized from water when cooled to 0 °C and a monohydrate[11c] from aqueous solution in the presence of an inorganic additive disodium terephthalate. A dihydrate phase of paracetamol was also reported, but was formed exclusively under high pressure conditions.[12] Peterson et al[11b] commented that paracetamol trihydrated

CrystEngComm Accepted Manuscript

form appeared to be maximally solvated species with limited thermal stability. The porosity introduced by the expulsion of water from the trihydrated structure caused a density difference and resulted in the collapse of the structure to a more favorable anhydrous structure. The direct phase transformation of trihydrate to form I was thought to be fundamentally different from that of the metastable form III of paracetamol which rather required two step phase transformations, first to form II and then to form I. Interestingly, when we performed in-situ Raman mapping experiments on freshly prepared trihydrate samples, we found an evidence for a transient phase on the route of trihydrate to its more stable form I. We present these results in this manuscript.

Experimental Section

Materials and methods: Paracetamol was purchased from Sigma-Aldrich. The white crystalline powder was characterized by DSC and PXRD [Fig. S1 of Electronic Supplementary Information (ESI)]. Its melting point at 169 ^{o}C and a good resemblance of the powder XRD pattern to the simulated pattern from the monoclinic crystal structure[8a] confirmed it as crystalline form I. There were no extra peaks in the diffraction pattern which confirmed the material to be pure with in the detection limits (>99.5%).

Paracetamol trihydrate form preparation: The trihydrate form of paracetamol was prepared using the method described by Peterson et al.[11b] Approximately 30 mg of paracetamol was dissolved in 1mL of water on the hot plate. Aqueous solutions of dissolved paracetamol were brought to room temperature to cool for 2-3 minutes and were quickly quenched by immersing the vials or conical flasks in the liquid nitrogen. Samples were carefully transferred to a micro slide using the spatula and the frozen ice gently melted, leaving lath crystals of paracetamol in the cold aqueous solution (Fig.S2 of ESI). Micro Raman spectra were collected on the lath crystals using the confocal set up. The acquired spectrum nicely matched with the reported Raman spectrum by Peterson et al[11b] and indicated the lath crystals to be trihydrate form of paracetamol. The crystals were found to undergo a rapid dehydration in a few minutes at room temperature when they were separated from cold aqueous solution and the crystals turned opaque instantaneously. However, the trihydrate samples were relatively more stable and did not turn opaque when they were fully immersed under the cold aqueous solution. They showed a gradual solvent mediated phase transformation to the stable polymorph I. Optical images and Raman spectra were collected to confirm the phase transformation from trihydrate to form I (Fig.S3 and S4 of ESI).

Surface mapping experiments: In-situ Raman mapping was performed on one of the trihydrate samples. A rectangular area (800×600 μm) of trihydrate lath crystals under cold aqueous solution was identified and mapped with 4,781 gird points. Raman spectrum at each grid point was acquired using the confocal Raman microscope. An image of the identified area was created by employing statistical routines on the acquired spectral data. Time interval images, optical (Fig.1) and Raman maps (Fig.2), were recorded at 20, 40, 60, 80 and 100 minutes. The trihydrate sample showed two phase transformations, first to form X and then to form I. When the time resolved in-situ mapping on the trihydrate sample was about to finish, we were interested in mapping a different region of same sample, because we noticed the formation of "fine needles, blocks and hexagons" along the periphery of the sample (Fig. S5). We wanted to understand the spectral differences of these three crystal shapes. Single point Raman spectra were collected. Blocks and hexagon corresponded to form I, whereas, fine needles are form X. Following this, we recorded two time interval Raman maps in this peripheral region (please refer to Fig. S5 and S6 of ESI) to capture the phase transformation events of form X to I and to confirm its transient nature.

Confocal Raman Microscopy: The Raman microscope from HORIBA Jobin Yvon (LabRam HR) with high quality filters discarded much of the Rayleigh scattering and facilitated the access to the low-wavenumber region (20-400 cm^{-1}) and intramolecular region (400-4000 cm^{-1}). The Raman instrument was calibrated with the Rayleigh line at 0 cm^{-1} and on standard silicon with peak position at 520.7 cm^{-1}. An Olympus BX51 microscope was used with a 50× magnification lens to focus the laser on the sample. A green laser of 532 nm wavelength was employed. 600 lines/mm grating was selected. Dispersed signals were detected by a 1024 pixel synapse CCD detector which was thermoelectrically cooled at -65 ^{o}C. The standard deviation for a given peak is noted to be with in the acceptable limit (±0.1 cm^{-1}) and other systematic errors are estimated to be within the typical range for Raman spectroscopy measurements.[3b] Raman spectra of reference materials in the wavenumber range 20-4000 cm^{-1} were collected using the multi-window mode, in which the grating position was moved and required more time for spectral data acquisition. However, for in-situ Raman mapping, the spectra should be collected rapidly, for which a single-window mode was used, in which, the grating position was fixed at 890 cm^{-1} to collect the spectra in the wavenumber range 20-1800 cm^{-1}. The data acquisition time for spectrum at each point was set to 0.1 sec. The spectra were collected using the high speed electron multiplying charge coupled device (EMCCD) detector. The confocal aperture was set to 150μm for obtaining good spatial resolution.

Data Analysis: LabSpec software version 5.54.15 (Horiba Jobin Vyon, Japan)[13a] generated a single ".ngc" output file containing all the spectra collected in each map. The combined output file was converted to ".txt" format. The spectral data were normalized in the statistical software R,[13b] where each spectrum was mean-centered and divided by its root mean-square, which is typically known as auto scaling of the data, using the standard "scale" function in the base package of R. The multivariate curve resolution (MCR) method[13c] was employed to deconvolute the Raman mapping data. For this, a separate MCR-ALS package

CrystEngComm Accepted Manuscript

This journal is © The Royal Society of Chemistry [year]

(alternating least-squares algorithm) was installed in R and routine data processing treatments like median centering and variance scaling were performed. In the mapped data presented in Fig.2, the initial guesses for MCR-ALS were obtained from a pile of randomly generated numbers.[6a] A good correlation between the identified loadings and the reference spectra is seen (Fig.2). Other approaches like Principal Component Analysis (PCA)[13d,e] or PCA(SVD)[13f] can also be employed for obtaining initial guesses in an unknown material. We employ the PCA approach for analyzing the mapped data of the peripheral region consisting of "needles, blocks, and hexagon" (Figure S5 and S6 of ESI), and was found to be better in defining the spectral profiles correctly, compared to the random numbers approach.[6a] To carry out principal component analysis (PCA) on the spectroscopic data, a separate PCA library module was installed in R and the normalized spectroscopic data were given as input. The first four principal components accounted for 98% of the variance of the data, and the PCA output result was used accordingly in the MCR-ALS algorithm. False color images of MCR scores plots were generated in Ctioga version 2 0.2.[13g] The plotting software XmGrace[13h] was used comparing the spectra of various solid forms of paracetamol.

Results and Discussion

The trihydrate form of paracetamol could be prepared in sufficient quantities using the flash-cooling of aqueous solution of paracetamol by immersing the sample in liquid nitrogen, as reported previously.[11b] Lath-crystals characteristic of trihydrate form of paracetamol[11a,b] were obtained (Fig. S2 of ESI). The majority of trihydrate samples were found to transform to form I (Fig. S3 and S4 of ESI) as noted earlier by McGregor et al[11a] and Peterson et al.[11b] Interestingly, one of the trihydrate sample with relatively smaller lath crystallites (<100µm, Fig. 1) compared to the larger laths (>500µm, Fig. S2 and S3 of ESI) showed two step phase transformation - first to form X and then to form I. The results are discussed now.

The time interval optical images of trihydrate sample are presented in Fig.1 and the corresponding Raman images recorded at 20, 40, 60, 80 and 100 minutes are presented in Fig.2. For in-situ mapping, a rectangular area of the trihydrate sample (800×600µ dimension) was selected and mapped with 4,781 Raman spectra at a spatial resolution of 10µm between the two adjacent spectra. The mapping was performed at room temperature. A single map required approximately 20 minutes time. The spectral data matrix was analyzed by statistical software R using the MCR-ALS module,[13c] which decomposed the data matrix into spectral profile (Loadings) and concentration profile (Scores). The distribution of the phases in the MCR scores plot is shown using the pseudo colors (Fig. 2). Red color points to areas where the phase is present to its maximum and black color indicates areas where the phase is present to its minimum/absent. Sample with varying concentration are shown by continuum of colors from red to black.

The MCR analysis on the trihydrate sample at 20 minutes is presented in Fig.2a. A single component, MCR1 loading, is required to deconvolute the spectral data matrix, which nicely matches with the reference spectrum of trihydrate form. The entire area in scores plot is identified in red color indicating the whole of the surface corresponds to trihydrate form. In the next two maps, after 40 minutes (Fig. 2b) and 60 minutes (Fig. 2c), the MCR analysis is similar to the 20 minutes map. The trihydrate form is solely identified as MCR1 loading. However, the MCR analysis at 80 minutes (Fig. 2d) shows interesting observations. Three components are required instead of one and the analysis identifies three different phases of paracetamol, clearly indicative of phase transformations. The first component, MCR1, is identified as form I of paracetamol, while the subsequent MCR2 and MCR3 recognize trihydrate and a new phase "X", respectively. On spectral comparison, the new phase X is found to be different from other known polymorphs II, III and amorphous material. The location in which the trihydrate material transformed to new form X was identified and a single point Raman spectrum was recorded on that sample area to obtain a reference spectrum for new phase X and compared with MCR3 loading in Fig. 2d. In further time mapping experiment at 100 minutes (Fig.2e), a single component is indicated and the identified MCR1 loading matches the reference spectrum of form I. The scores plot also indicates that the whole of the surface is actually defined by form I only. Nevertheless, MCR analysis was attempted with three components assuming there could be some trace amount of trihydrate and new form X, but the MCR analysis indicated their absence. The trihydrate and new phase identified in 80 min map underwent a clear phase transformation to form I in 100 min map. No other phase transformations were noticed beyond this point. The existence of a transient phase on the route of trihydrate to form I is clearly evidenced in this experiment.

In a different location of the same trihydrate sample (please see experimental section), we noticed the formation of fine needles, hexagons, and blocks of paracetamol (Fig.S5 of ESI). Out of curiosity, single point Raman spectra were collected on all crystal shapes. Fine needles were identified as transient phase X, hexagons and blocks as form I. Because, the form X needles were found completely outside the aqueous solution, we could obtain the whole range Raman spectra (20-4000 cm^{-1}) using multi-window mode to make a spectral comparison with known forms of paracetamol. The transient nature of form X was reconfirmed in the time interval Raman maps (Fig. S5 and S6 of ESI). Needles of form X rapidly transformed to form I, however, their morphology was maintained even after its phase transformation.

Spectral comparison of form X with other solid forms of paracetamol (Fig. 3) indicates that there are distinguishable features both in the phonon and molecular region. In the phonon region, a single broad peak is seen for amorphous form whereas multiple characteristic peaks are seen for various crystalline forms (Table 1, Fig. 3a). Form X exhibits

CrystEngComm Accepted Manuscript

a distinct pattern from other forms. In the molecular region (Fig.3b and Fig.3c), form X shows unique peaks at 1237.9 and 1320.2 cm^{-1} of phenyl ring vibrational modes. Besides, there are clear peak shifts in other molecular vibrations (Table 1). The red shift of C=O stretching frequency in form X (a shift towards a lower frequency value) compared to other forms perhaps suggest its involvement in stronger hydrogen bonding.

The N-H and O-H stretches are well separated for crystalline forms in the region 3100-3500 cm^{-1} showing subtle differences in the peak positions, accounted by different crystal environments (Fig. 3d). However, such a clear discrimination between N-H and O-H bands is not seen for the disordered amorphous form. In the trihydrate form, broad doublet peaks in the region 3100-3500 cm^{-1} are seen due to interference of water O-H bands with paracetamol N-H and O-H stretches, as the spectrum was recorded on trihydrate crystals when fully immersed in aqueous solution. Attempts to dry the trihydrate crystals by removing them from solution and placing on the filter paper resulted in a rapid transformation to form I. A unique O-H stretching is seen in the transient phase X at 3470.1 cm^{-1}, in addition to peaks at 3303.2 cm^{-1} for N-H and 3168.8 cm^{-1} for hydroxyl O-H, which clearly suggests an extra O-H band from water inclusion in the crystal. As the form X needles were well outside the water, the interference from water O-H is tentatively ruled out. The transient phase X is therefore indicated to be a hydrated form, different from the parent trihydrate.

The formation of a monohydrate of paracetamol[11c] was reported under ambient conditions, for which the Raman spectrum was not available to make a direct comparison. Unfortunately, our efforts to prepare the monohydrate form using the reported procedure with an inorganic additive[11c] led to the thermodynamically stable polymorph I. We have also attempted to record a time resolved PXRD measurements on trihydrate samples to capture the transient phase. However, the trihydrate material when separated from aqueous solution for PXRD measurements, underwent a quick transformation to form I. Similar conclusions were reached earlier by Peterson et al.[11b] Based upon the observed needle like morphology of the form X crystals (Fig. S5 of ESI), we suggest that they most likely correspond to the monohydrate, which was reported to be needle-like by Parkin et al.[11c] We do not however completely rule out the phase transformation via other possible transient phase, such as new stoichiometry hydrate. We also compared form X with the Raman spectra of two new phases of paracetamol observed under high pressure by Smith et al[14] and found it to be spectrally different. Further experiments are in progress to grow suitable crystals and identify the transient hydrated phase by single crystal X-ray diffraction. The results nonetheless demonstrate the capabilities of in-situ Raman mapping in identifying a transient phase of paracetamol on the way of trihydrate material's transformation to anhydrous polymorph I.

Conclusions

Time resolved in-situ Raman mapping experiments were performed on rapidly converting trihydrate samples of paracetamol. To analyze thousands of spectral data collected during mapping experiments, statistical routines MCR-ALS and PCA were employed to deconvolute the whole spectral data matrix into few representative phases in each of the map. At 20, 40, 60 minutes of time mapping, the whole surface could be represented by the trihydrate form. At 80 minutes of mapping, trihydrate sample showed interesting observations. Three different phases of paracetamol were identified in the MCR analysis. The first component, MCR1, is identified as form I, while subsequent MCR2 and MCR3 are identified as trihydrate and new phase X of paracetamol, respectively. In further time resolved experiments, the trihydrate and new phase X disappeared completely and indicate their unstable nature. At 100 min map, only a single MCR component represented the entire spectral data and that component matched with the form I of paracetamol. The phase transformation events are noticed in the order, trihydrate → transient phase X → form I. Noteworthy to mention that, the statistical routines employed in this present study are unsupervised and identified all paracetamol phases clearly. A previous study dealing with the trihydrate form of paracetamol[11b] thought of a possible intermediate form on its way of transformation to the stable form I, but was directly not observed in their study. We provide the first experimental evidence in support of this view in this present study and demonstrate the capabilities of in-situ Raman mapping in identifying transient phases.

Acknowledgements

We thank the Nottingham Nanotechnology and Nanoscience Centre (NNNC) for providing an access to the Raman microscope and the East Midland Development Agency (EMDA) for funding this equipment. JBN and JCB thank the EPSRC for financial support under grant EP/G038740/1.

Notes

[a]Laboratory of Biophysics and Surface Analysis (LBSA), School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, United Kingdom (UK).
E-mail: jonathan.burley@nottingham.ac.uk

[$]Raman Imaging on the other hand, requires an image of the sample to be focused onto an array detector, where the intensity of the radiation passing through each region of the sample is measured at each pixel. The signal at a given wavelength is recorded at each pixel and the image from one wavelength region is measured at all pixels simultaneously.[7a]

[†]Electronic Supplementary Information (ESI) available: [DSC and PXRD patterns of paracetamol form I, optical

CrystEngComm Accepted Manuscript

This journal is © The Royal Society of Chemistry [year]

images of lath shaped trihydrate crystals, solvent mediated phase transformation confirmed from optical images and Raman spectra, time interval optical and Raman maps of the peripheral region of trihydrate samples].

References

1) J. Haleblian, W. McCrone, *J. Pharma. Sci.*, 1969, **58**, 911.
2) (a) J. Bernstein, Polymorphism in Molecular Crystals, Clarendon, Oxford, 2002; (b) H. G. Brittan, Polymorphism in Pharmaceutical Solids, Marcel Dekker, New York, 1999.
3) (a) R. Hilfiker, Polymorphism in the Pharmaceutical Industry, Wiley-VCH, Weinheim, 2006; (b) S. Ekins, Pharmaceutical Applications of Raman Spectroscopy, S. Šašić (Ed.), John Wiley & Sons, 2008.
4) (a) S. Al-Dulaimi, A. Aina, J. Burley, *CrystEngComm*, 2010, **12**, 1038; (b) A. Brillante, I. Bilotti, R. G. D. Valle, E. Venuti, A. Girlando, *CrystEngComm*, 2008, **10**, 937; (c) A. P. Ayala, *Vib. Spectrosc.*, 2007, **45**, 112; (d) S. Roy, B. Chamberlin, A. J. Matzger, *Org. Proc. Res. Dev.*, 2013, **17**, 976; (e) P. J. Skrdla, D. Zhang, *J. Pharm. Biomed. Anal.*, 2014, **90**, 186; (f) A. C. Williams, V. B. Cooper, L. Thomas, L. J. Griffith, C. R. Petts, S. W. Booth, *Int. J. Pharm.*, 2004, **275**, 29; (g) D. S. Hausman, R. T. Cambron, A. Sakr, *Int. J. Pharm.*, 2005, **299**, 19.
5) (a) S. Stewart, R. J. Priore, M. P. Nelson, P. J. Treado, *Annu. Rev. Anal. Chem.*, 2012, **5**, 337; (b) S. Šašić, Y. Ozaki, Eds. Raman, Infrared, and Near-infrared Chemical Imaging, Wiley, John Wiley & Sons, 2011; (c) U. Gala, H. Chauhan, *Expert. Opin. Drug. Discov.*, 2014, doi: 10.1517/17460441.2015.981522.
6) (a) F. Tres, K. Treacher, J. Booth, L. P. Hughes, S. A. C. Wren, J. W. Aylott, J. C. Burley, *J. Control. Rel.*, 2014, **188**, 53; (b) J. B. Nanubolu, J. C. Burley, *Mol. Pharm.*, 2012, **9**, 1544; (c) S. Piqueras, L. Duponchel, R. Tauler, A. de Juan, *Anal. Chimica. Acta.*, 2014, **819**, 15; (d) S. Hellstén, H. Qu, T. Heikkilä, J. Kohonen, S. Reinikainen, M. Louhi-Kultanen, *CrystEngComm*, 2012, **14**, 1582; (e) J. Breitenbach, W. Schrof, J. Neumann, *Pharma. Res.*, 1999, **16**, 1109; (f) A. Alkhalil, J. B. Nanubolu, C. J. Roberts, J. W. Aylott, J. C. Burley, *Cryst. Growth Des.*, 2011, **11**, 422; (g) A. Brillante, I. Bilotti, R. G. D. Valle, E. Venuti, M. Masino, A. Girlando, *Adv. Mater.*, 2005, **17**, 2549; (h) f) S. Šašić, S. Mehrens, *Anal. Chem.*, 2012, **84**, 1019; (i) J. C. Burley, A. Alkhalil, M. Bloomfield, P. Matousek, *Analyst*, 2012, **137**, 3052; (j) P. Matousek, A. W. Parker, *J. Raman Spectrosc.*, 2007, **38**, 563.
7) (a) R. Salzer, H. W. Siesler, Eds. Infrared and Raman spectroscopic imaging, WILEY-VCH, Weinheim, 2009; (b) C. Huang, H. Hamaguchi, S. Shigeto, *Chem. Commun.*, 2011, **47**, 9423; (c) A. Bonifacio, C. Beleits, F. Vittur, E. Marsich, S. Sameraro, S. Paoletti, V. Sergo, *Analyst*, 2010, **135**, 3193; (d) A. Daniel, A. Prakasrao, B. David, L. Joseph, C. M. Krishna, D. Koteswaran, S. Ganesan, *J. Raman Spectrosc.*, 2014, **45**, 541.
8) (a) M. Haisa, S. Kashino, R. Kawai, H. Maeda, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1976, **32**, 1283; (b) T. N. Drebushchak, E. V. Boldyreva, *Z. Kristallogr.*, 2004, **219**, 506; (c) M. A. Perrin, M. A. Neumann, H. Elmaleh, L. Zaske, *Chem.Commun.*, 2009, 3181; (d) J. C. Burley, M. J. Duer, R. S. Stein and R. M. Vrcelj, *Eur. J. Pharm. Sci.*, 2007, **31**, 271.
9) (a) P. Di Martino, P. Conflant, M. Drache, J. -P. Huvenne and A. -M. Guyot-Hermann, *J. Therm. Anal.*, 1997, **48**, 447; (b) S. Qi, P. Avalle, R. Saklatvala, D.Q. Craig, *Eur. J. Pharm. Biopharma.* 2008, **69**, 364; (c) P. Di martino, G. F. Palmieri, S. Martelli, *Chem. Pharm. Bull.*, 2000, **48**, 1105.
10) (a) T. Wu, L. Yu, *Pharma. Res.*, 2006, **23**, 2350; (b) L. Zhu, L. Wong, L. Yu, *Mol. Pharma.*, 2008, **5**, 921; (c) L. Zhu, J. Jona, K. Nagapudi, T. Wu, *Pharma. Res.*, 2010, **27**, 1558; (d) T. Wu, Y. Sun, N. Li, M. M. de Villiers, L. Yu, *Langmuir*, 2007, **23**, 5148.
11) (a) P. A. McGregor, D. R. Allan, S. Parsons, C. R. Pulham, *J. Pharm. Sci.*, 2002, **91**, 1308; (b) M. L. Peterson, D. McIroy, P. Shaw, J. P. Mustonen, M. Oliveira, Ö. Almarsson, *Cryst. Growth Des.*, 2003, **3**, 761; (c) A. Parkin, S. Parsons, C. R. Pulham, *Acta. Crystallogr. Sect E. Struct. Rep. Online*, 2002, 58, o1345.
12) F. P. A. Fabbiani, D. R. Allan, W. I. F. David, S. A. Moggach, S. Parsons, C. R. Pulham, *CrystEngComm*, 2004, **6**, 505.
13) (a) LabSpec software 5.54.15 by Horiba Jobin Vyon, 2008, URL http://www.horiba.com/scientific/products/raman-spectroscopy/software/functionality/ (b) R Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2011, ISBN 3-900051-07-0, URL http://www.R-project.org/ (c) K. M. Mullen, ALS: multivariate curve resolution alternating least squares (MCR-ALS). R package version 0.0.5, 2014; URL http://cran.r-project.org/web/packages/ALS/ALS.pdf (d) P. J. Turner, XMGRACE, Version 5.1. 19. *Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton, OR* (2005), URL http://plasma-gate.weizmann.ac.il/Grace/ (e) I. Jolliffe, *Principal component analysis*, John Wiley & Sons, Ltd, 2002. (f) J. Jaumot, A. de Juan, R. Tauler, Chemom. Intell. Lab. Sys., 2015, **140**, 1; (g) V. Fourmond, ctioga2, the polymorphic plotting program, 2007-2014, URL http://ctioga2.sourceforge.net/index.html (h) W. Stacklies, H. Redestig, M. Scholz, D. Walther, J. Selbig, *Bioinformatics*, 2007, *23*, 1164; URL http://www.bioconductor.org/packages/release/bioc/html/pcaMethods.html
14) S. J. Smith, M. M. Bishop, J. M. Montgomery, T. P. Hamilton, Y. K. Vohra, *J. Phys. Chem. A*. 2014, **118**, 6068.

CrystEngComm Accepted Manuscript

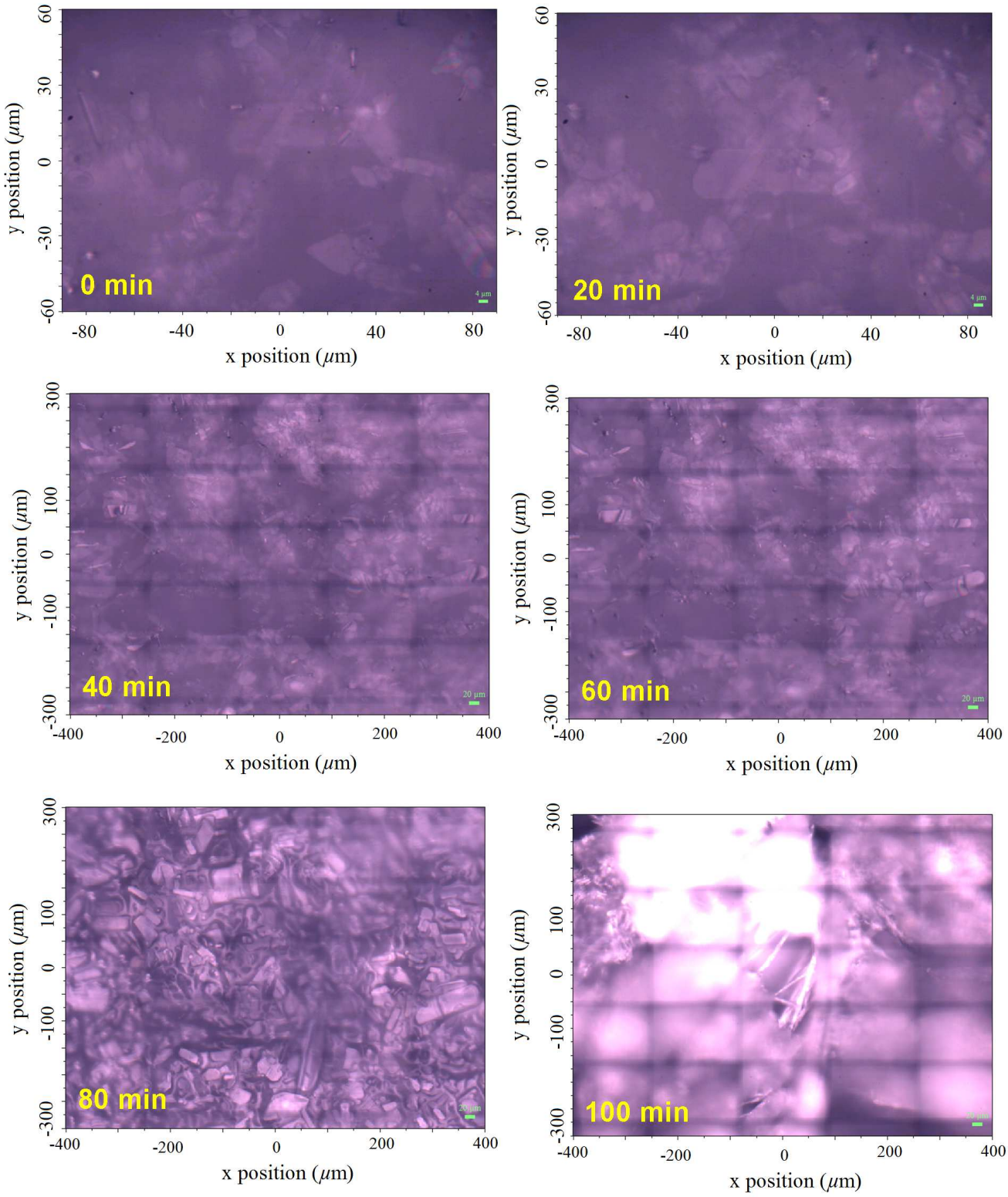


Fig. 1. Time interval optical images of trihydrate sample recorded at 0, 20, 40, 60, 80, 100 minutes. Images were captured using the extended video feature available in the Labspec software.[13a]

CrystEngComm Accepted Manuscript

This journal is © The Royal Society of Chemistry [year]

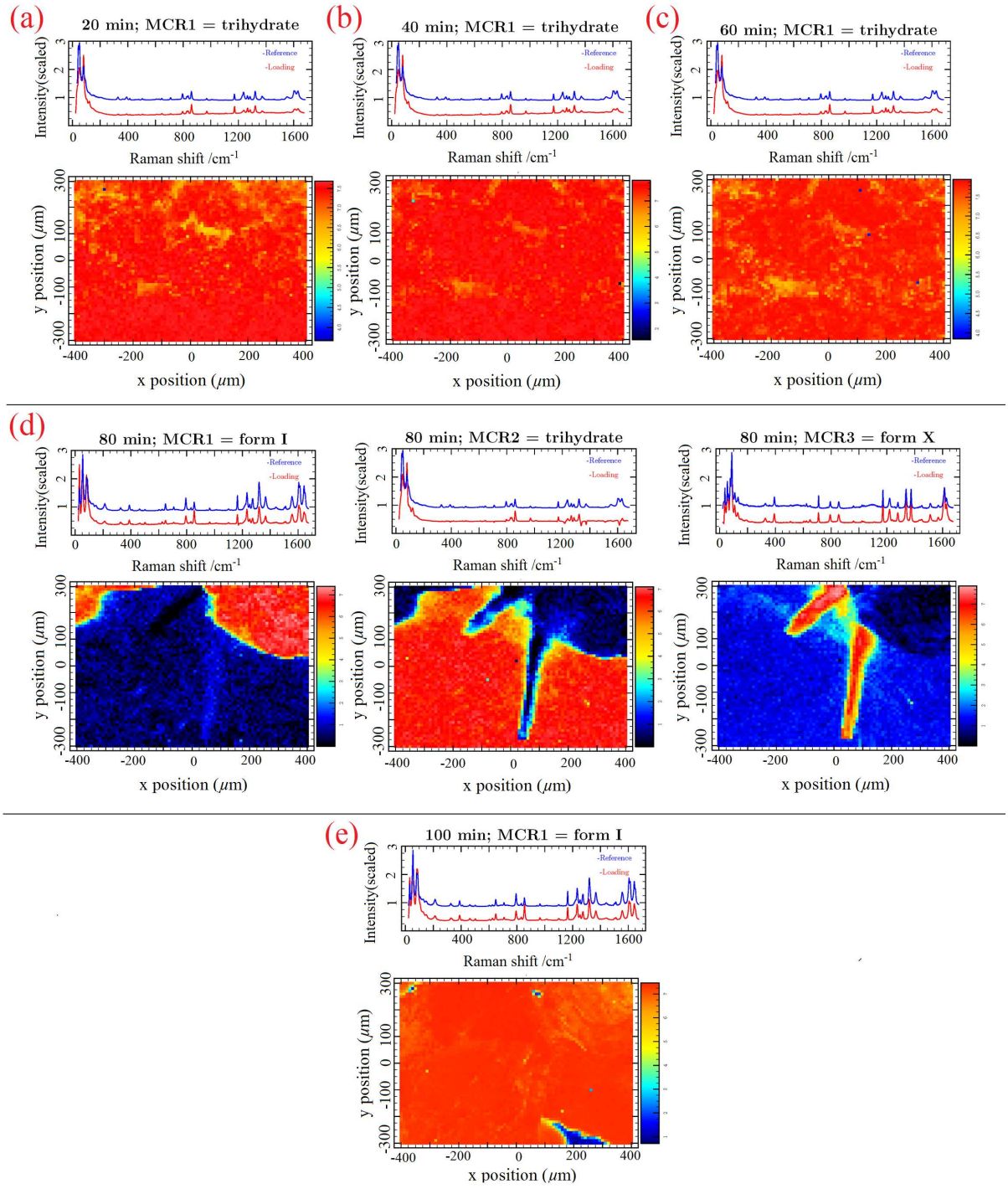


Fig. 2. Time interval Raman images on a rectangular surface area (800 ×600μ dimension) of the trihydrate sample. MCR loadings represent the spectral features of solid forms and MCR scores plot shows the distribution of solid forms on the mapped surface. The first row images represent MCR analysis of maps at 20 minutes (Fig. 2a), 40 minutes (Fig.2b) and 60 minutes (Fig. 2c). A single component was required in all of them to deconvolute the spectral data. The MCR loading matches the reference spectrum of trihydrate material. The second row images represent MCR analysis at 80 minutes (Fig. 2d). Three components are required for spectral deconvolution. The first component, MCR1, is identified as form I, while subsequent MCR2 and MCR3 are identified as trihydrate and transient phase (form X) of paracetamol, respectively. Third row image represents MCR analysis at 100 minutes map (Fig.2e). Both trihydrate and transient phase show complete transformation by this time. The identified MCR1 loading matches the reference spectrum of form I.

CrystEngComm Accepted Manuscript

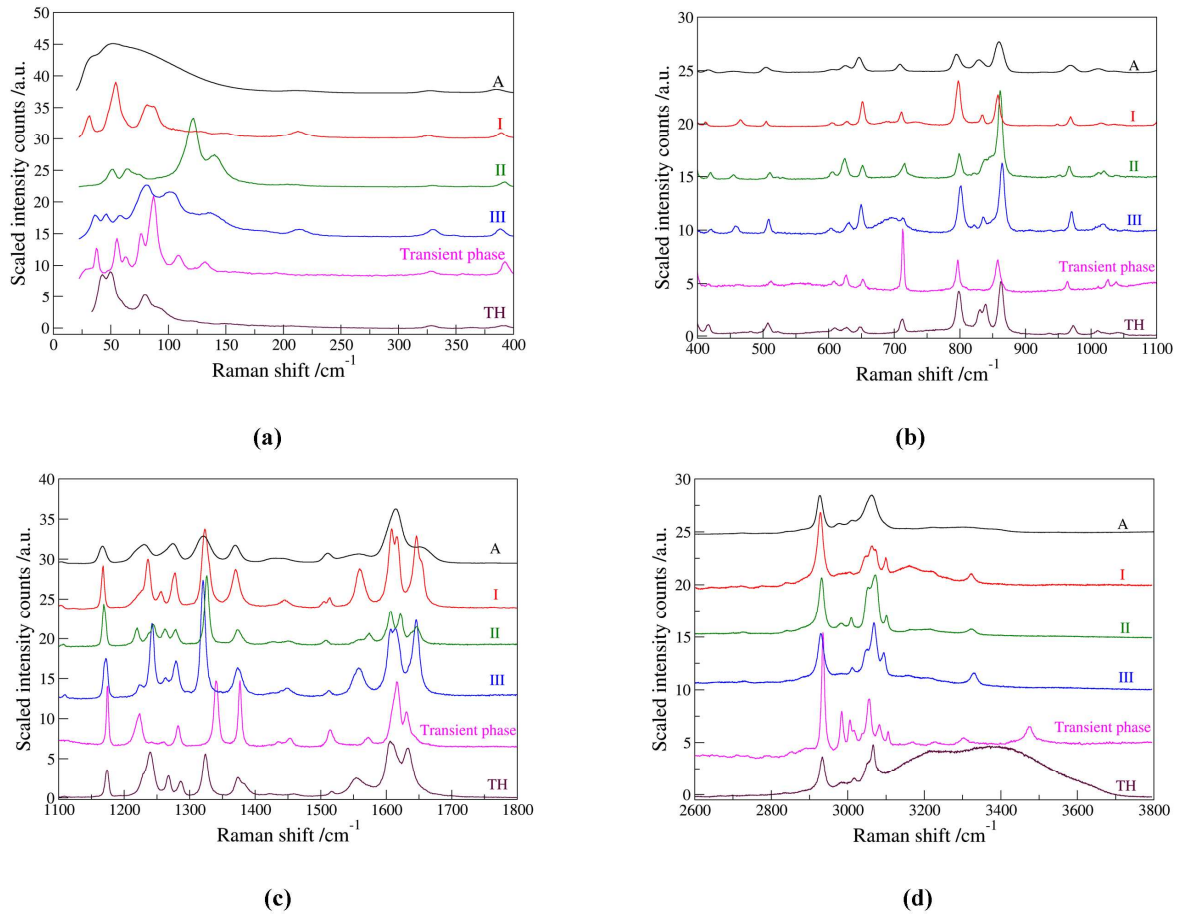


Fig.3. Spectral comparison of transient phase X with five other forms of paracetamol, illustrated separately for (a) 0-400 cm^{-1}, phonon region; (b) 400-1100 cm^{-1}, finger print region; (c) 1100-1800 cm^{-1}, finger print region; (d) 2600-3800 cm^{-1}, X-H region. Labels A, I, II, III, TH point to amorphous, crystalline forms I, II, III and trihydrate of paracetamol, respectively.

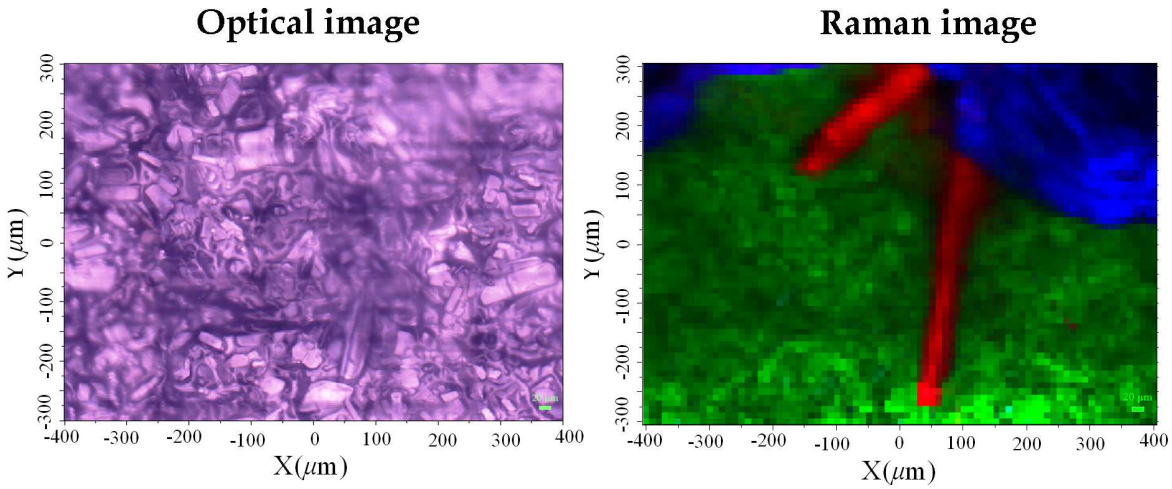
Table 1. Important vibrational modes of paracetamol observed in various solid forms. All peak positions are referred in cm^{-1} unit scale.

	Form I	**Form II**	**Form III**	**Trihydrate**	**Transient Form X**	**Amorphous**
Lattice or phonon vibrations	31.5, 54.2, 81.4	50.8, 64.4, 122.0, 140.0	36.1, 46.3 57.6, 81.4 136.6	42.5, 49.3, 79.9, 90.9	37.3, 55.2 62.2, 76.8 86.9, 108.5 132.1	Broad peak 51.8
C=O stretching	1646.1	1647.0	1646.1	1633.8	1630.9	1651.0
N-H bending	1558.9	1574.1	1558.0	1554.2	1572.2	1558.7
C=C stretches	1608.3, 1616.8	1607.3, 1621.5	1606.4, 1613.0	1606.4	1615.9	1614.3
N-H and O-H stretches	3323.1, 3161.0	3321.6, 3162.6	3329.2, 3150.9	Broad doublet peak 3209.3, 3427.4	3303.2, 3168.8, 3471.9	Broad peaks from 3100-3500

CrystEngComm Accepted Manuscript

This journal is © The Royal Society of Chemistry [year]

Graphical Abstract



In-situ Raman surface mapping on trihydrate sample provides an evidence for the existence of a transient phase X on the route to its stable anhydrous polymorph I. The phase transformation events are observed in the order, trihydrate → transient phase → form I.

CrystEngComm Accepted Manuscript