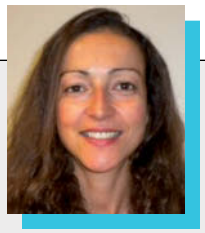

Ligia Bras

Polymorphic conversion monitoring using real-time Raman spectroscopy

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KEYWORDS

PAT (Process Analytical Technology); Raman spectroscopy; polymorphism; crystallization.

ABSTRACT

This article reports the application of in-line Raman spectroscopy as a PAT (Process Analytical Technology) tool to monitor a urea formation at pilot scale. This reaction was critical since it involved the formation of different polymorphic forms with distinct solubility properties, which impacted the overall process yield and purity. Raman spectroscopic data collected during the reaction allowed the tracking of polymorphic conversion, providing better insight into the process and offering a faster analytical response than conventional in-process testing by X-ray powder diffraction. Once integrated in an appropriate control strategy, this PAT tool enabled an enhanced process step control and helped achieve the targeted product purity and yield.

INTRODUCTION

Since polymorphs first identification the search for methods to monitor and control their formation has been of great interest in the pharmaceutical industry. The existence of different polymorphic forms of a product may allow a patent extension as the polymorphic forms of an active pharmaceutical ingredient (API) are patentable. Typically, polymorphic forms occur when products are crystallized or precipitated from a reaction mixture and the analytical techniques most commonly used to identify them are X-ray powder diffraction (XRPD), Infra-red or Raman spectroscopy and Differential Scanning Calorimetry (DSC) (1). Both XRPD and DSC analyses are usually carried out off-line and are not likely to have a fast response (average time of analysis is one hour). On the other hand, Raman spectroscopy is a fast and accurate analytical technique that allows effective monitoring and control of industrial processes, pursuing the concept of an innovative state in Pharmaceutical Manufacturing where PAT and Quality by Design (QbD) are applied (2-8). The concept and terminology QbD have become part of the common language of those working in the pharmaceutical industry. Guided by the ICH documents Q8, Q9, Q10 and more recently Q11 (4-7), this initiative aims to bring the industry to a higher level of innovation and process control through a better process understanding and thus to develop improved processes with positive implications in terms of process capability, sustainability and cost-efficiency.
Sharing with infrared spectroscopy its selectivity and specificity, Raman spectroscopy is defining its own niche in the pharmaceutical industry as a versatile technique, with applications such as raw materials identification, detection of counterfeit drugs, trace analysis, solid state analysis, reaction kinetics and reaction monitoring (9-12). Raman spectroscopy is based on an inelastic scattering of monochromatic light sourced from a laser in the visible, near infrared or near ultraviolet range. The Raman spectrum of a molecule will be specific to the chemical bonds and symmetry of the molecules, thus providing a chemical fingerprint that can be used in qualitative and quantitative applications. Continued advancements in fibre optics technology, instrumentation and miniaturized sensors address some of Raman technology's drawbacks and made remote sensing capabilities for Raman spectroscopy feasible and cheaper, boosting the number of Raman applications in process analytics (10-12). Furthermore, it is possible to bring this technology to the process environment and apply it transversally in lab, pilot and industrial scale.
In this article, in-line Raman spectroscopy was applied at an early development stage to monitor polymorphic conversion observed at laboratory and pilot scale, during a urea formation. In this reaction, the urea crystallizes in a given polymorphic form and before the reaction end-point it is converted into a different one. This polymorphic conversion could not be easily controlled by adjusting crystallization conditions.

POLYMORPHIC CONVERSION DURING A UREA FORMATION REACTION

During the process development of an API under a QbD framework, it was determined that it was critical to control the polymorph formation during one of the intermediate chemical steps, since it affected overall process yield and purity (8). This particular step consisted of a urea formation via the reaction of an aromatic amine and a carbamate (Figure 1). The product precipitated during the chemical reaction and changing reaction conditions did not ensure that the same polymorphic form was consistently obtained. Therefore, it was desirable to have a real-time monitoring tool that allowed a deeper process understanding and a potential control over the polymorphic form obtained.

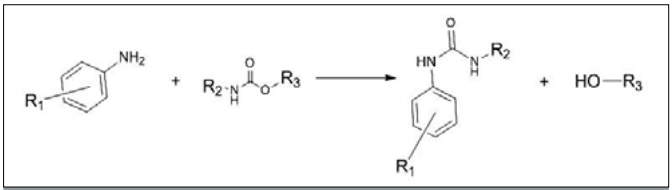

Figure 1. Urea formation by reaction of an aromatic amine and a carbamate.

Batch number	Polymorph	% Yield (molar)	Purity % area
1	I	90	99.3
2	II	93	98.9
3	III	76	99.0

Table 1. Production outcomes for the urea formation in different production batches: polymorphic form, yield and purity.

Solid state characterisation indicated that three different polymorphic forms (Form I, II and III) can be formed during this crystallization. Formation of Form III led to lowest yield and purity. It has a higher solubility in the reaction solvent than the other two forms, causing higher losses of product in the mother liquors and facilitating impurities precipitation (see Table 1). Based on the purity and yield obtained, Form I was identified as the preferred form. During the course of the reaction, Forms II and III are initially formed, and in time convert to the single Form I. Therefore, stopping the reaction too early during manufacturing might lead to the presence of Form III which results in product out-of-specification due to the reduced purging capacity of Form III. Hence, an off-line in-process control by XRPD was performed during this manufacturing step to guarantee that Form III is not present before product isolation. Additional in-process testing is carried out by HPLC to monitor the starting material consumption.

USE OF IN-LINE RAMAN SPECTROSCOPY

Given the critical impact of polymorph formation during this reaction on overall process yield and purity, it would be important to monitor and control the reaction in real-time. Work performed at laboratory scale using Raman spectroscopy showed that both product and starting raw materials had distinct characteristic bands which allows their fast identification and quantification (Figure 2). Crystalline Form II can be easily discriminated from the other two polymorphic forms, I and III, while there are a few spectral ranges that allow differentiation of Form III from Form I, specifically the presence of a small band at around 1717 cm^{-1} in Form III (Figure 2). Therefore, Raman spectroscopy was assessed as a potential in-line sensor tool to monitor polymorph formation and conversion in real-time with the objective of having it integrated in an appropriate control strategy and thus enhance process step control.
The use of the in-line Raman spectroscopy allowed a fast and efficient data collection during the urea formation, which led to the tracking of the polymorphic conversion (Figure 3 and Figure 4). At the same time, it was also possible to monitor the starting raw material consumption, which currently is determined using the off-line, time consuming, HPLC testing.
Plotting the intensity counts at specific frequencies of the Raman scattering spectra against time it was possible to follow the starting material conversion and identify the polymorphic transformation events taking place during the crystallization process as depicted in Figure 3, where:

- Starting material's consumption is tracked using the peak intensity at around 1553 cm^{-1}, adjusted by single-point (at 1525 cm^{-1}) baseline subtraction in order to correct for fluorescence baseline effects;
- Peak intensity at around 1564 cm^{-1} adjusted by single-point (at 1575 cm^{-1}) baseline subtraction in order to correct for fluorescence baseline effects, is used to monitor the formation of both Forms I and III;
- Peak intensity at around 1546 cm^{-1}, adjusted by single-point (at 1525 cm^{-1}) baseline subtraction in order to correct for fluorescence baseline, is employed to track Form II.

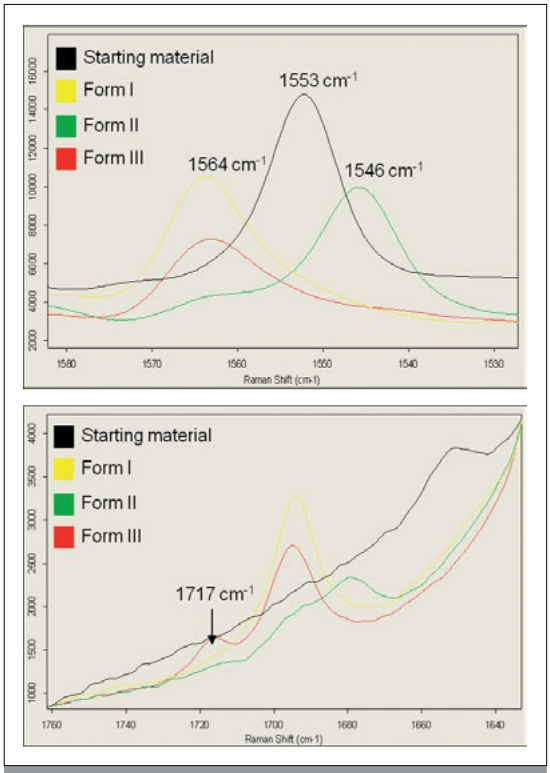

Figure 2. Raman spectra of the starting material and the identified polymorphic forms.

The pressure changes associated to sampling events are visible in the profiles as steep variations in the intensity measurements.
As indicated in Figure 3, the manufacturing process includes a seeding step that involves addition of crystalline Form I as laboratory trials demonstrated that the seed presence accelerates polymorphic conversion of Form II and III into Form I. Raman profiles indicate that Form II is the main polymorphic form present before seeding. After seeding with Form I, the intensity of the band attributed to Forms I and III starts to increase, while Form II intensity band decreases as this polymorph is being converted to Form I or Form III. To clarify the polymorphs' identity after seeding (Form I or III), the region comprising the band at around 1717 cm^{-1} was inspected. Intensity counts at the region between 1740 cm^{-1} and 1660 cm^{-1} were corrected for fluorescence baseline effects using a "two-point" baseline subtraction at these two frequencies. A three-dimensional plot at this selected

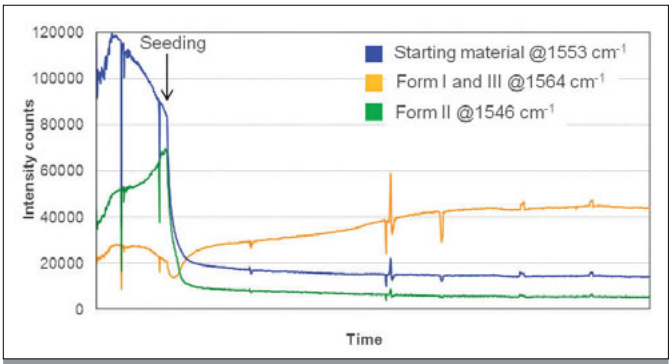

Figure 3. Raman-based profiles for the evolution of starting material and polymorphs along the reaction step.

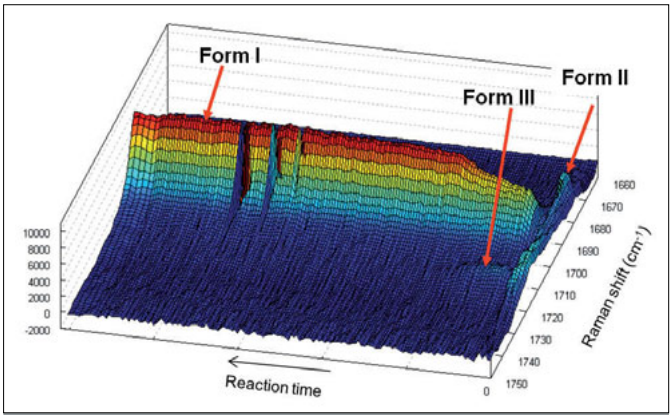

Figure 4. 3D display of the evolution of the Raman signal along reaction time.

region (Figure 4) shows that Form III is the one being formed immediately after seeding, but then converts to Form I, which is the final form obtained as confirmed by XRPD analysis. Implementation of in-line Raman technology improved process understanding and allowed control of this critical process step. It generated critical and timely information about starting material consumption and polymorph conversion which potentially eliminated the need for off-line testing by HPLC and XRPD. Additional benefits are analytical workload reduction and a decrease in handling of potent compounds by operators. Integrating this PAT tool in the process control strategy will result in an improved and robust process, which are the main goals of applying PAT.

EXPERIMENTAL SECTION

The Raman system employed in this work was a 4-channel Raman Analyzer (model RXN2C-785 from Kaiser Optical Systems, Inc.) equipped with fibre-coupled probe optic technology for in-situ process monitoring. A WetHead probe with a 12 inches-long SS316 immersion shaft and a sapphire optical window (short fixed focus) was utilized. The Raman system was also equipped with a MR probe head, a general laboratory probe head for non-contact sampling analysis or in-situ analysis using immersion optics.
Solid samples were analyzed using the non-contact MR probe head using one spectral accumulation with an exposure time of one second. The reaction was monitored using the process WetHead probe; spectra were acquired in the range of 3425 to 100 cm^{-1} using a resolution of 1 cm^{-1} and the auto-adjust exposure option with a target total exposure time of 15 seconds. A new spectrum was collected every minute. The spectrometer's software interface iC Raman (Mettler-Toledo) was utilized for instrument configuration, data acquisition and export. Data analysis was carried out in OPUS QUANT Spectroscopy Software (version 6.0) after exporting the raw Raman spectra to *spc* file format.
Spectra were pre-treated in order to minimize variability unrelated to the chemical entities of interest and to amplify the Raman scattering signal. In the case of Raman spectroscopy, the main factors that impact the spectra are the presence of fluorescence baseline effects and laser power fluctuations (13). Fluorescence effects typically can be noticed as a broad band convoluted with the Raman signal. On the other hand, laser power fluctuations affect the Raman signal, especially when monitoring long processes, and translate in an overall loss of Raman signal intensity.

CONCLUSION

Having informative real-time measurements during chemical reactions is a key enabler to more efficient processes development and is an attainable goal by suitable selection and implementation of PAT. In this article, in-line remote-sensing Raman technology was utilized to monitor polymorphic conversion of a urea in real-time, offering an in-situ PAT tool for enhanced process understanding. This tool also enables the implementation of a QbD approach in the development of more robust API manufacturing processes. Using this technology, it was possible to control the polymorphic form obtained in the urea reaction and improve product quality and yield, avoiding downstream processing issues.

REFERENCES AND NOTES

1. *Polymorphism in the Pharmaceutical Industry*, Edited by Hilfiker R., Wiley-VCH, Weinheim, Germany (2006).
2. *Pharmaceutical cGMPs for the 21st Century - A Risk-Based Approach*, Final Report, US FDA (2004).
3. *Guidance for Industry: PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*, US FDA (2004).
4. ICH Q8(R2): *Pharmaceutical Development*, ICH (2009).
5. ICH Q9: *Quality Risk Management*, ICH (2005).
6. ICH Q10: *Pharmaceutical Quality System*, ICH (2008).
7. ICH Q11: *Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)*, ICH (2012).
8. Matos N., Gil M., Brás L. and Loureiro R., *Speciality Chemicals Magazine*, 10-13 (2013).
9. *Infrared and Raman Spectroscopy, Methods and Applications*, Edited by Schrader B., VCH, Weinheim, Germany (1995).
10. *Analytical applications of Raman spectroscopy*, Edited by Pelletier M.J., Blackwell Publishing (1999).
11. Chowdhry B.Z., Jabeen S. and Alexander B., *European Pharmaceutical Review*, **5**, 34-39 (2009).
12. Das R.S. and Agrawal Y.K., *Vibrational Spectroscopy*, **57**, 163-176 (2011).
13. Romero-Torres S., Huang J. and Hernandez P., *American Pharmaceutical Review*, 12-19 (2009).

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