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Raman Spectroscopy as a Tool for Process Analytical Technology

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Abstract

Raman spectroscopy is a well-suited spectroscopic technique for process development and control within development laboratories in chemical, pharmaceutical and other industries. This article demonstrates the utility of portable Raman spectroscopy as a versatile tool for process analytical technology (PAT) for raw material identification, *in-situ* monitoring of reactions in developing active pharmaceutical ingredients (APIs), and for real-time process monitoring. Raw material identification is done for verification of starting materials as required by PIC/S and cGMP, and can be readily done with handheld Raman. Portable Raman systems allow users to make measurements to bring process understanding and also provide proof of concept for the Raman measurements to be implemented in pilot plants or large-scale production sites. For known reactions which are repetitively performed or for continuous online process monitoring of reactions, Raman provides a convenient solution for process understanding and the basis for process control.

Introduction

Process analytics has been in use for nearly 70 years, starting in the petrochemical and chemical industries utilizing infrared photometers and devices such as oxygen and conductivity sensors as process analyzers to control manufacturing and refining processes (1). These univariate sensors and others continue to be used even as multivariate sensors including spectroscopic systems are also adopted. Process analytics can provide the pulse of a process and is important in quality product manufacturing, from the receipt of raw material and development of reaction processes all the way through to full-scale production. Process analytical technology (PAT) is the use of off-line, at-line, or in-line analyzers to obtain analytical data faster, more often, and with high precision to increase manufacturing controls of biological or chemical materials as well as aid in process understanding for optimization and improvement (2). In the pharmaceutical industry, interest in PAT has grown since the 2004 publication of the US FDA guidance on PAT utilization in the pharmaceutical industry. PAT is more than the measurement of a process, as it is intended to ensure consistent product, and therefore begins at the verification of the raw material used in manufacturing. Spectroscopic tools including Raman can be used *in-situ* or be interfaced to a sampling loop on a process to monitor the chemical composition with full spectral information, giving a complete molecular picture of the changes occurring in the process. The amount of data that can be collected may exceed the speed of a process, which, in the early phases of process development, can be used to understand a process and its dynamics, possible side reactions, and kinetic pathways. The ability to control processes to make consistent product through data verification during production and process understanding and control is recognized in the 2012 guidance from the European Medicines Agency on Real-Time Release testing. (3)

Raman Spectroscopy for Raw Material Identification

Raman spectroscopy is a laser-based form of molecular spectroscopy that provides specificity and sensitivity for qualitative and quantitative analysis of substances through their molecular vibrations. It is used in many different environments as an analytical tool for the study of solids, liquids, slurries and gases. Handheld instrumentation can be used for the nondestructive identification of incoming raw materials. The technique has the ability to make measurements through transparent packaging, minimizing the need for sampling and providing rapid identification. Raman has high selectivity and specificity for identification of many organic and inorganic compounds including typical solvents, excipients, and active ingredients. The Raman spectrum of different polymorphs of a material are often quite distinct, as the molecular vibrations vary with the arrangement of the molecules, allowing for easy discrimination. An example of this is given in Figure 1, where the Raman spectra of alpha-lactose and beta-lactose are shown as measured with the handheld NanoRam® from B&W Tek.

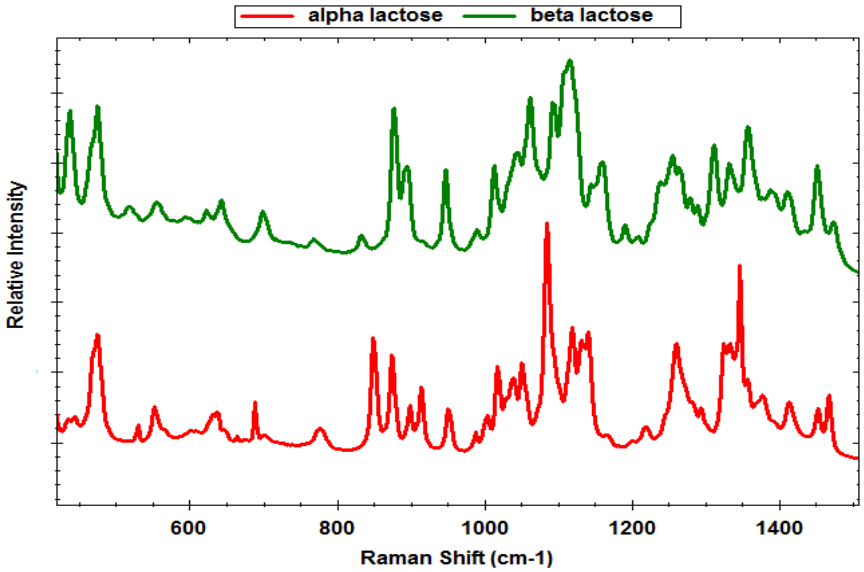

Figure 1: Raman spectra of two polymorphic forms of lactose: alpha-lactose and beta-lactose.

Raman Spectroscopy for Process Monitoring

Many Raman instruments are interfaced with a fiber-coupled probe, which gives the versatility and flexibility for measurements to be made in different places, including *in-situ* as is often the requirement for the monitoring of processes. Over a decade ago, hardware developments that led to an increase in the adoption of Raman spectroscopy were recognized as the development of compact lasers, introduction of spectroscopic-grade charge-coupled device detectors, improvements in sampling optics (including fiber-optic probes) and the advances in powerful personal computers and associated software to collect and analyze volumes of data.(4) These, as well as the advancement of technology in laser and spectrometer miniaturization and improved filters for laser light rejection and fiber-optic probes, have allowed for

development of portable Raman spectroscopy systems that can be deployed and transported to different locations for uses that include process analysis. There are other complementary spectroscopic techniques to Raman such as FTIR and NIR, but due to Raman spectroscopy's flexible sampling interface, high sampling rate and high spectral specificity, Raman is an invaluable tool for qualitative and quantitative analysis of chemical systems for reaction monitoring and end-point detection for chemical synthesis and polymerization reactions (5), hydrogenation, hydrolysis and polymorphic characterization. The range of pharmaceutical unit operations which Raman has been applied for PAT includes product design, API synthesis, formulation, crystallization, milling, blending, granulation, drying, tableting, packing, and final product verification. (6,7)

The application of PAT tools early in the process development provides process understanding and aids in the development of robust processes that can consistently produce quality products. In development of a process, there are many parameters that may be evaluated and experiments performed to optimize the yield, purity, and cycle time. To understand the impact of process parameter changes on the process and product, *in–situ* measurements can be made that relate the process parameters to product properties. In choosing measurement tools in process development, the needs of the chemical system in terms of "purpose, specificity, sensitivity, cycle time, on-line/off-line, qualitative/quantitative, accuracy, precision" must be defined and the proper technology that can fulfill the defined criteria must be chosen. (8,9) At early phases of process development, qualitative information on reaction progress may be sufficient to gauge reaction completion. The temporal evolution of the reaction can be determined from trending of reactant and product relative concentrations based on peak height or area in a spectrum can be used to follow a reaction, even as other reaction parameters, such as solvent and other reagents or temperature, are changed from one reaction to another. In reactions where stoichiometric control is required, a quantitative measure of reactant concentrations may be needed. Off-line measurements by HPLC can be made and quantitative calibration models developed, but such models are matrix dependent and often require updating or redevelopment when the reaction matrix is changed.

We will present an example of Raman applied in process development and also an example of quantitative monitoring of a full-scale crystallization process. In both cases portable Raman spectroscopy was used, giving the versatility to use equipment in a process development laboratory and move it between labs to monitor different reactions as the development work continued, and to transfer the equipment to monitor the full-scale process on plant.

Experimental details for process understanding of synthesis of API

The goal of this study was to determine the end point of the chemical reaction for the 2-phenylimidazo[1,2-a]pyridine synthesis. This compound was under development as the API for medicinal chemistry, and traditional thin-layer chromatography (TLC) was used to verify reaction completion. Typically very few aliquots are taken during small-scale reactions, and timing for sampling for the end-point is based on the visual precipitation in the reaction mixture followed by the TLC test. Information on reaction progress, possible formation of intermediates and side products is possible only when Raman spectra are collected

regularly throughout the course of the reaction. Monitoring the reaction with *in-situ* Raman spectroscopy reduces the need for removing samples for analysis and also provides real-time information on reaction progress.

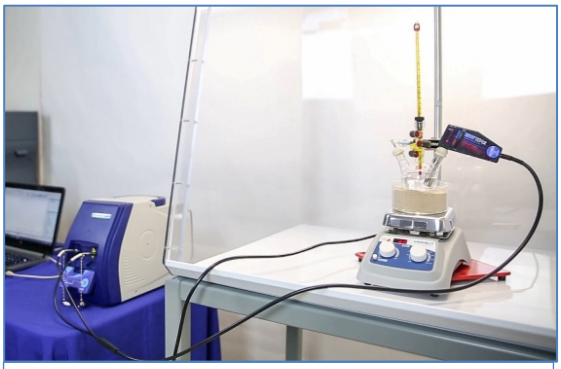

Figure 2: Portable i-Raman® Plus spectrometer with fiber-coupled immersion probe inserted in reaction

The experimental setup consisted of an i-Raman® Plus portable Raman spectrometer from B&W Tek with a 785nm, 300mW laser excitation source connected to a spectrograph equipped with a back thinned charge-coupled device (CCD) array detector covering a spectral range from 65cm^{-1}-3200cm^{-1}. The sampling interface was a fiber optical bundle with an immersion probe to allow for *in-situ* measurements during the reactions. The Raman instrument was used to monitor the synthesis 2-phenylimidazo[1,2-a]pyridine. The immersion probe was inserted into the round bottom flask for direct measurement throughout the course of the reaction as seen in Figure 2. The starting reagents were suspended into acetonitrile and a small amount of sodium bicarbonate was added to neutralize the hydrobromic acid that formed during the process. The reaction was placed under argon atmosphere and heated to 80° C for approximately two hours. Raman spectra were collected every minute during the reaction with an integration time of three seconds and coaddition of ten spectra.

In this S_n2 chemical reaction for development of a medicinal chemistry compound, the goal of the process monitoring was to gain understanding of the reaction and to detect the reaction end-point. In this synthesis 2-aminopryidine is reacted with 2-bromoacetophenone to form 2-phenylimidazo[1,2-a]pyridine. To monitor the reaction, a univariate approach of monitoring the reactant and product peaks was conducted using B&W Tek's BWSP® software.

For the reaction of the synthesis of 2-phenylimidazo[1,2-a]pyridine, a spectrum was taken for each of the starting materials and final product to identify the Raman peaks to monitor during the course of the reaction. As shown in Figure 3, the three regions of interest are 847cm^{-1} (2-aminopyridine, reactant 1), 1547cm^{-1} (final product) and broad dual peaks between 1684-1702cm^{-1} (2-bromoacetophenone, reactant 2). Because of the specificity of the Raman spectrum and the fact that these identified peaks do overlap with other Raman peaks of solvent or other reaction components, univariate analysis can be effectively used. During the progression of the reaction the two product peaks at 1547cm^{-1} and 1603cm^{-1}, respectively were monitored as shown in Figure 4.

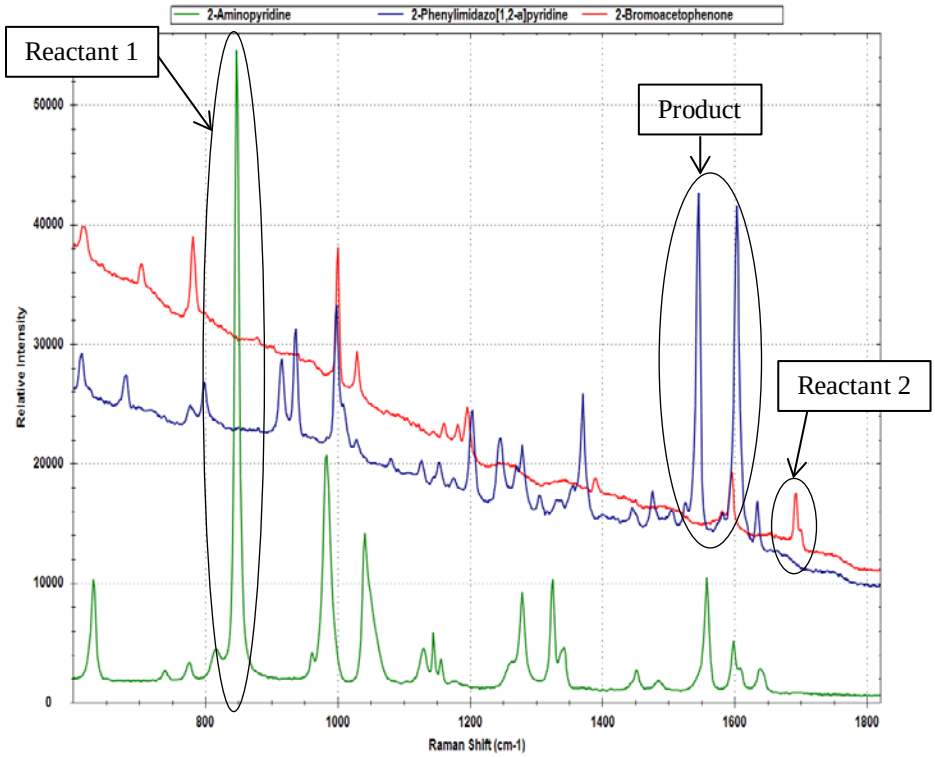

Figure 3: Overlay of the Raman spectra of the reactants and product for the first synthesis, Green is 2-aminopyridine (reactant), Red is 2-bromoacetophenone (reactant), and Blue is Final Product: 2-Phenylimidazo[1,2-a]pyridine.

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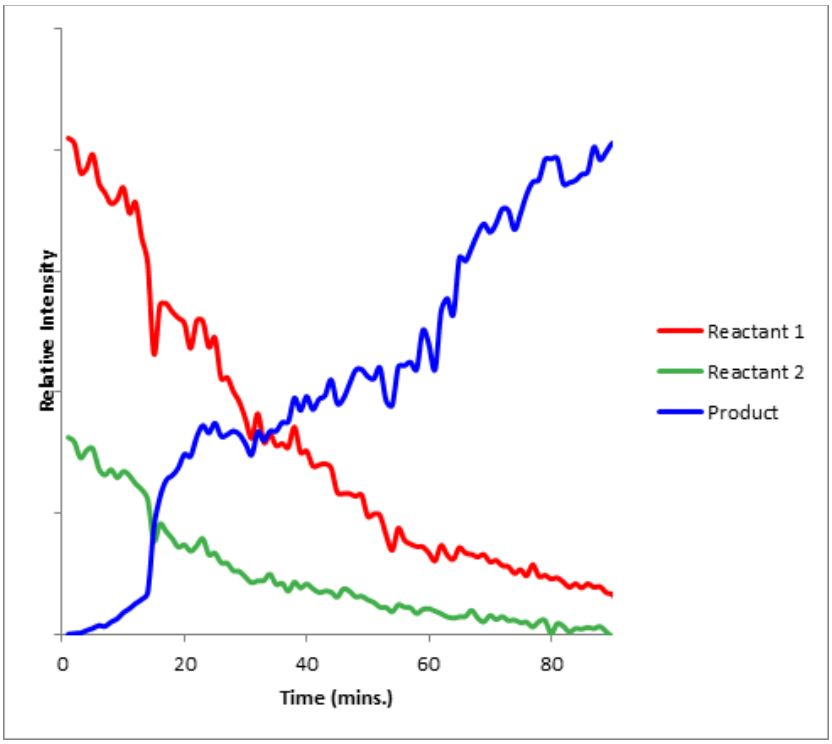

Figure 4: Peak trending of the two reactant and product peaks.

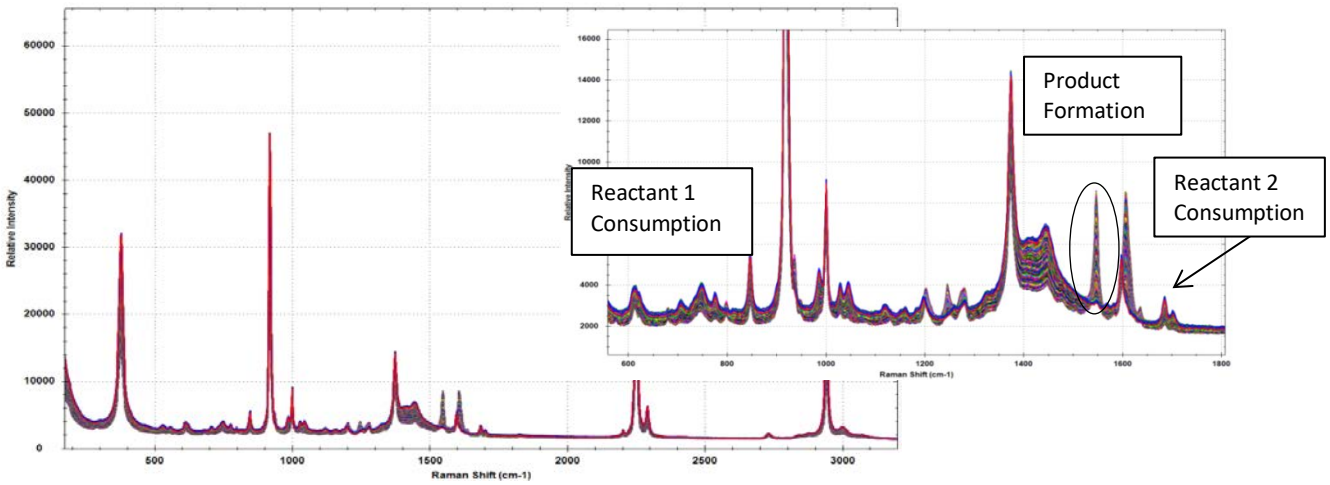

Figure 5: Raman reaction monitoring data: note the consumption of the two reactant peaks at 847cm^{-1} (reactant 1) and the broad band between 1684cm-1702cm^{-1} (reactant 2) as well as the formation of the product at 1547cm^{-1}.

The Raman spectra collected throughout the reaction monitoring are shown in Figure 5, with the reactant peaks visibly diminishing while the product peak is simultaneously increasing as the reaction progresses.

The univariate analysis provided supportive information about the end point of the reaction as shown in Figure 6 where the overlay of the first and last spectrum from the reaction illustrates the complete consumption of the reactants. Based on the peak trending plots of the reactants and product along with the overlay the first and last spectra collected, Figure 6, the reaction appears to finish within 2 hours. The measurement of several runs showed this to be consistent, and indicate that Raman can be used for reaction end-point detection.

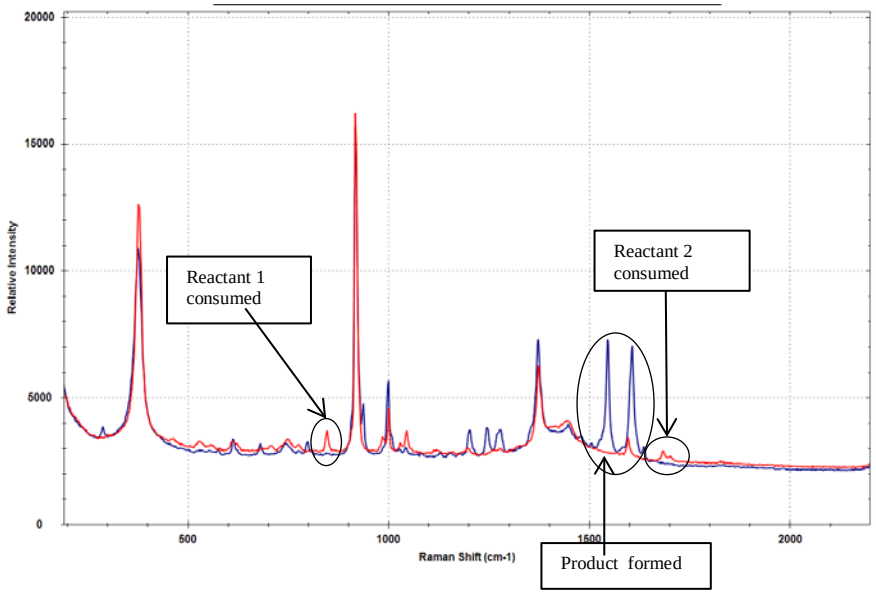

Figure 6: Overlay the first and last spectra of the reaction giving evidence of consumption of reactants and product formation.

Experimental details for Quantitative Monitoring of Crystallization

Real-time measurements are used to improve the process efficiency and product quality of boric acid, and important product in boron refinement operations. The concentration of sodium sulfate and boric acid must be controlled to optimize the process while minimizing production costs. If the levels of the these two components are outside of this range, the process produces large amounts of waste, greatly impacting the process costs. The sodium sulfate level is typically in the range of 22-34%, with the most efficient level being 30%, just below the solubility limit of 31.8%. The aqueous crystallization process of borate and sulfuric acid for the formation of boric acid and sodium sulfate was monitored using the portable i-Raman® Plus with industrial immersion probe was interfaced to process flow line. The Raman, with a 785 nm laser excitation was operated at 350 mW power, and data collected with 3 second integration time, with 10 averages. As the process is lengthy, spectra were collected hourly. The instrument was housed in a NEMA enclosure, providing a stable operating environment of 25 ± 2°C, and protection from water and dust.

Quantitative models for the concentration of the products were developed using partial least square (PLS) regression in the BWIQ® chemometric software. Raman spectra of 80 production samples were measured on the portable Raman in the laboratory and reference values for the boric acid and sodium sulfate

determined using titration methods, providing reference values. The instrument was then installed on the process and further data collected close to the process target concentration. The reaction was sampled and additional reference values measured by titration to augment the PLS calibration models. Figure 7 shows the best fit of the PLS regression model for the sodium sulfate which has a target concentration range of 22-34%. The Raman spectral data were baseline corrected and normalized and PLS regression computed over the spectral region that includes the 993 cm^{-1} symmetric stretch vibration of the sulfate functional group shown in Figure 8.

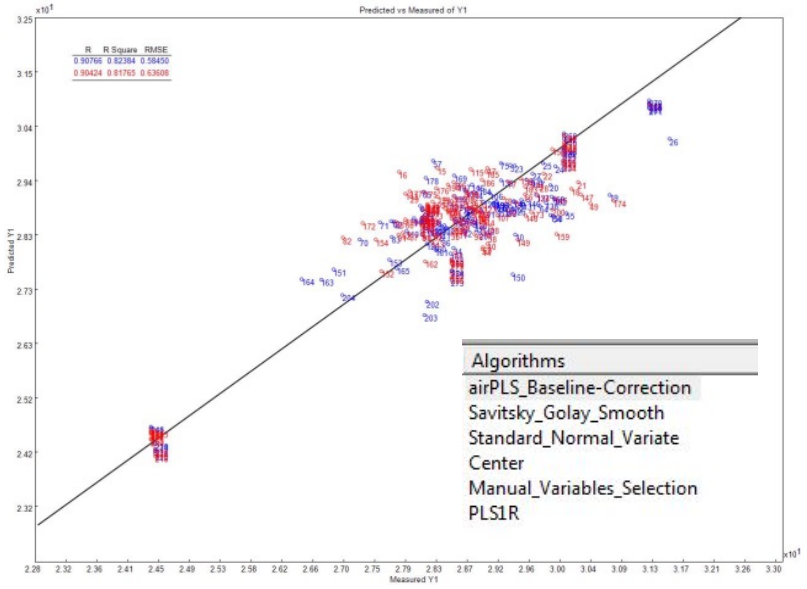

Figure 7: PLS Regression model for % Na_2SO_4 in the crystallization process.

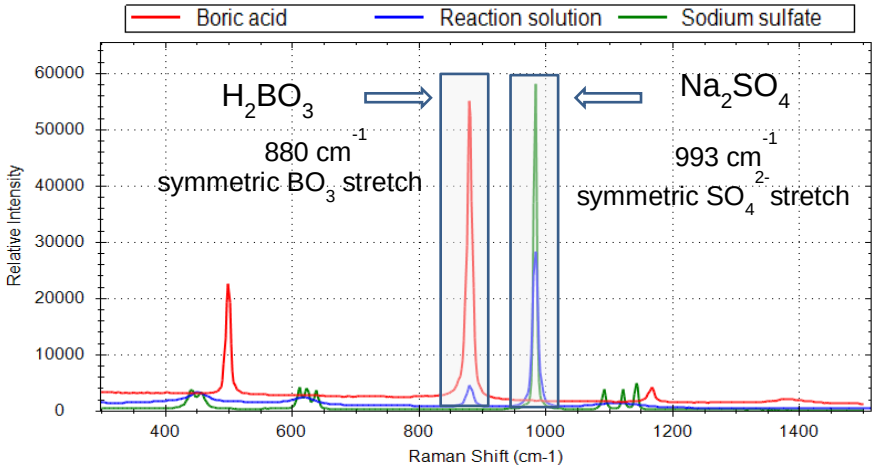

Figure 8: Raman Spectra of product and reactants of crystallization process.

The portable Raman system facilitated the development of initial calibration models in the laboratory using retained production samples. The system was then installed on plant and the calibration augmented before being used to successfully monitor the process.

The Raman- predicted values for the sodium sulfate level over the course of one month of production is shown in Figure 9. The trend plot includes the limit lines of the target concentrations and the solubility limit. The process monitoring illustrates the fluctuations in the process, and also indicates that during this production period, the sodium sulfate levels were maintained below the solubility limit.

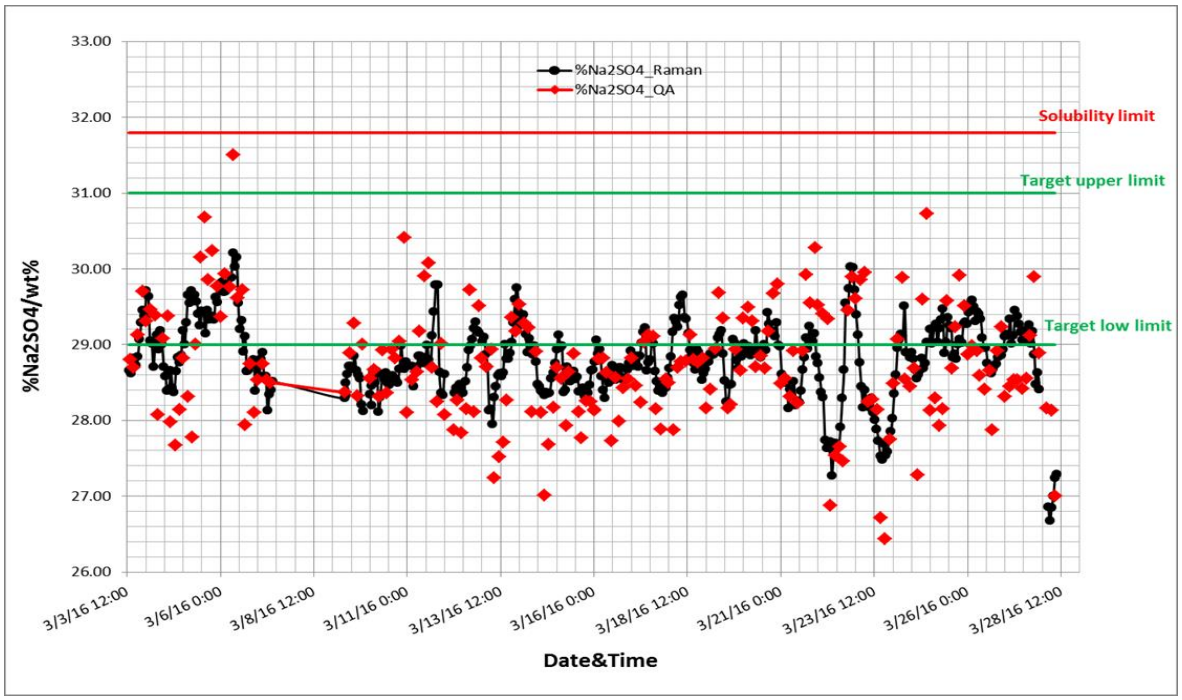

Figure 9: Online Raman predicted values for sodium sulfate as measured during one month, with the target values indicated.

Conclusions

Raman spectroscopy for process analytics is an invaluable tool from the inspection of incoming raw materials, the development of processes, and production process monitoring. It can provide information critical for understanding reactions that have significant benefits for the chemical, pharmaceutical and other industries. This work demonstrates the versatility and ability of portable Raman spectrometers and their utility in process development and understanding. Implementation of Raman spectroscopy at various stages of development and processes contributes to the ability to consistently manufacture quality products. By having better process understanding, key process parameters can be determined and operations developed with these well controlled.

In this work we demonstrated the ability of portable Raman spectroscopy in the process development stage to gain insight and process understanding of chemical reactions, specifically in determining reaction end-points, and on full quantitative process monitoring. The Raman spectral data was valuable to monitor a developing synthetic reaction qualitatively. An example of an on-line implementation of Raman spectroscopy on a full scale manufacturing process with quantitative measurement of crystallization products was also provided.

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Further Reading

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Portable Raman for Polymorphs and Polymorphic Transitions

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NanoRam Datasheet

