WHITE PAPER

Enhancing Pharmaceutical Formulation Development through the Use of High Performance Raman Spectroscopy.

Case Study – The Use of Tornado Raman for the Development and Verification of a Drug Delivery Strategy

Shaun Fraser, Colin Couper and Mark S. Kemper, Customer Success Group (CSG), Tornado Spectral Systems, Inc., Mississauga, ON Canada

ABSTRACT

Applying meaningful real time analysis techniques to novel drug delivery systems can often be challenging. However, the ability to verify the physicochemical properties of these formulations both in the development phase and during production is critical. With a delivery system that involves drug inclusion in a carrier molecule, the verification of that inclusion is vital to the maintenance of the pharmacological integrity of the formulation. The work shown in this paper suggests that Raman based spectrometers enabled by the High-Throughput Virtual Slit (HTVS) can be useful for *in situ* real time verification of the inclusion complex formation. This makes it a potentially valuable PAT tool for the verification of the proper manufacture of these formulations.

INTRODUCTION

Traditional administration of therapeutics is often limited in effectiveness by drug properties such as water solubility, permeability, and targeting specificity. To produce the desired pharmacological response, a specified *in vivo* level must be achieved, which is often limited by the solubility of the drug (Vemula et al 2010). Reduced drug solubility may require more frequent and higher doses to the patient, which can result in increased cost and lower drug effectiveness (Edward & Li 2008).

Increasing drug solubility can be achieved in a multitude of ways, such as reducing particle size, selecting and controlling the appropriate polymorphic form, performing chemical modifications, addition of solubilizers or using drug carriers.

Cyclodextrins (CDs) are a class of drug carriers that form an inclusion complex with insoluble molecules to increase solubility and impact greater pharmacological responses (Gareth 2007). CDs are oligosaccharides composed of six (α), seven (β), or eight (γ) glucose units that are concatenated to form a ring (seeFigure 1). The ring is effectively hydrophilic on the outside and hydrophobic on the inside, allowing the inclusion of hydrophobic molecules and creating a water-soluble CD-drug complex.

Many different analytical techniques can be used to confirm the inclusion of molecules into CDs, such as but not limited to; NMR spectroscopy (Betlejewska-Kielak et al 2021), DSC (Periasamy 2020), XRD (Kim 2020), FTIR spectroscopy (Crupi et al 2010), and Raman spectroscopy (de Oliveira et al. 2011).

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Raman spectroscopy has proven to be a suitable technology for investigating host-guest interaction of water-insoluble drugs into CDs as it is non-destructive, has a high degree of chemical specificity, is sensitive to polymorphic and crystalline structure changes, and can be used in- and on-line as a Process Analytical Tool.

Raman spectroscopy, however, is an energy-limited technique with approximately one in a million incident laser photons inducing a Raman photon scattered to a useful wavelength, limiting the technique's usage and practicality in various settings. With an order-of-magnitude improvement in efficiency over conventional slit spectrometers, Tornado Spectral System's HTVS™ technology addresses this limitation by allowing for 10x faster measurements and 3x or better improvement in the limit of detection compared to the best conventional process Raman spectrometers.

With the enhanced capability of the Tornado HyperFlux™ PRO Plus Raman spectrometer, we demonstrate that the technology can be used as an orthogonal analysis for drug inclusion complexes, provides molecular information related to the drug delivery system, and can be used to monitor the inclusion process *in situ*. By providing additional molecular information on the drug delivery system during *in situ* complexation, Raman spectroscopy can reduce production costs, enhance drug development efforts, and facilitate accurate process control for drug delivery systems.

EXPERIMENT AND DATA ANALYSIS

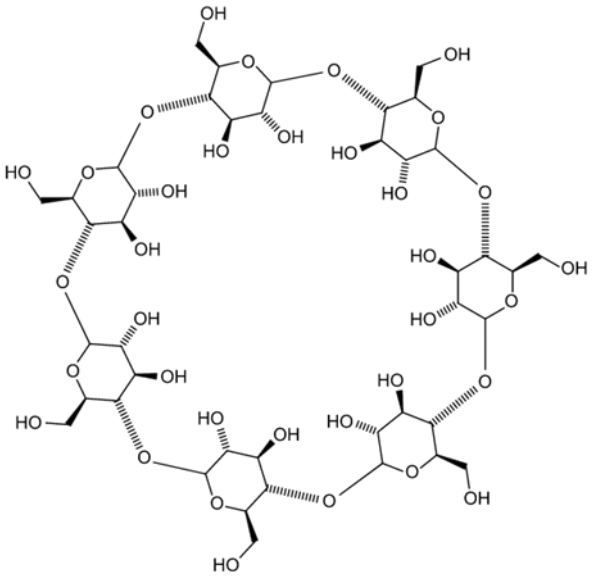

Figure 1: *Chemical structure of Beta cyclodextrin.*

Differential Scanning Calorimetry - The DSC thermograms were recorded on a SDT Q600 from QA instruments (Dallas, TX, USA). Each sample (3 to 5 mg) was heated in a crimped aluminum pan over a temperature range of 50°C to 200°C at a constant scanning rate of 5°C/min with nitrogen purging (20 mL/min). A blank aluminum pan was used as a reference.

Experimental Conditions - A Tornado HyperFlux™ PRO Plus Raman spectrometer equipped with a 785-nm laser was used for these experiments. Measurements were facilitated using a Tornado Hudson™ 50-mm standoff optic configured to measure through a 10-mm thick sapphire window into a custom-built high pressure (12,000 psi tolerance, 11 mL volume) vessel.

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Heat tape was wrapped around the vessel and a controller was used to bring the temperature of the system to 40 °C, and thus into the supercritical phase. Pressure was maintained at 2500 psi by a Dynesco high pressure syringe pump. A stir bar was added to the vessel with a stir plate underneath to effect mixing. Inclusion of CBD into CD was achieved using supercritical conditions over a period of 80 min. After completion of the inclusion process, the pressure was released to allow recovery. For the mechanical mixture, CBD and CD were mechanically mixed with a mortar and pestle without the use of solvents.

Materials - The representative insoluble drug used in this study was cannabidiol (obtained from a regulated online Canadian retailer) and a modified CD (Tokyo Chemical Industry Co). CD and CBD were added to the supercritical vessel at a 1:1 molar ratio (200 mg of CBD and 1200 mg of CD).

Raman Collection Parameters - The integration time of the spectrometer was set to yield a suitable spectrum every 1 min and 15 seconds (5000ms x 15 averages). The laser power was set to 495mW.

Data Analysis and Visualization - Data analysis and visualization were performed with PEAXACT multivariate analysis software from S-PACT GmbH.

MCR-ALS Analysis - **M**ultivariate **C**urve **R**esolution – **A**lternating **L**east **S**quares provides a bilinear decomposition of mixed experimental data into estimates of the chemically meaningful pure profiles of the respective chemical species. It is used to generate corresponding time-resolved pure component Raman spectra and relative scores/ratios of the pure components (Zhang et al. 2015). MCR-ALS was performed with PEAXACT from S-PACT GmbH using the *in situ* data obtained from monitoring the supercritical mixture experiment. The following were used for MCR-ALS analysis: 3 components, forced non-negative spectra and non-negative concentrations, concentration profiles with a single maximum, no forced sum of concentrations closure, maximum of iterations of 100, maximum 20 unsuccessful iteration attempts, and a convergence tolerance of 1e-05.

RESULTS

Figure 2 contains the DSC thermogram of the CBD/CD mixtures by the supercritical CO_2 method and the mechanical mixing method. The thermogram of the mechanical mixture (orange profile) shows an endothermic peak at 66.65°C, which is representative of CBD's melting point of 67.5°C. In the supercritical mixture method (gray profile), this endothermic peak has disappeared almost entirely. In its place there is now an exothermic peak at 215°C. The formation of an inclusion complex is suggested by the absence of the melting endotherm peak.

Raman spectra are shown in Figure 3 and Figure 4. Spectra are shown for CBD (yellow), for CD (red), for the mechanical mixture of CBD/CD (blue), and for CBD/CD after the complexation effected by the supercritical mixture method (green). Consistent with the DSC results, there are noticeable differences in the Raman spectra when comparing the mechanically mixed CBD/CD and the supercritical mixture of CBD/CD. There are changes in both peak ratio intensity and band shift. The carbonyl band of the cyclodextrin (1740 cm^{-1}) undergoes a shift to 1745 cm^{-1} and a band broadening. This may be interpreted as additional intermolecular forces and strain imposed on some of the carbonyls in the molecule. The band widening suggests that not all the carbonyls are undergoing this strain, which further implies that perhaps the fraction of CD carbonyls internal to the complex are under strain due to the included CBD molecule. For CBD, the most obvious change is the relative band ratio changes of 1626 cm^{-1} (benzene breathing/ C-C stretch), 1644 cm^{-1} (C=C alkene stretch), and 1663 cm^{-1} (C=C ring stretch) (Socrates 2001). The intensities of the bands related to the ring and alkene stretches are decreased relative to the band represented by the benzene breathing mode. This further suggests that the CBD is included into the CD. Other diagnostic changes can be found in the C-H stretch region to additionally corroborate the changes seen in the fingerprint region (Figure 4).

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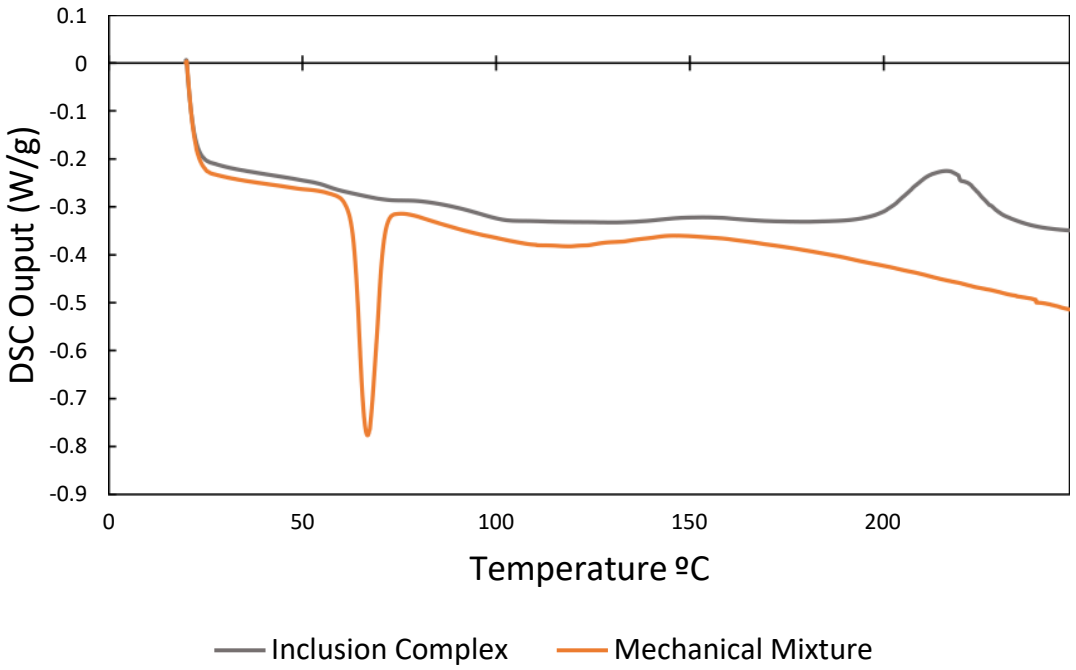

Figure 2: *DSC thermogram of the CBD/CD mixtures created by the supercritical CO_2 inclusion method (grey) and the mechanical mixing method (orange).*

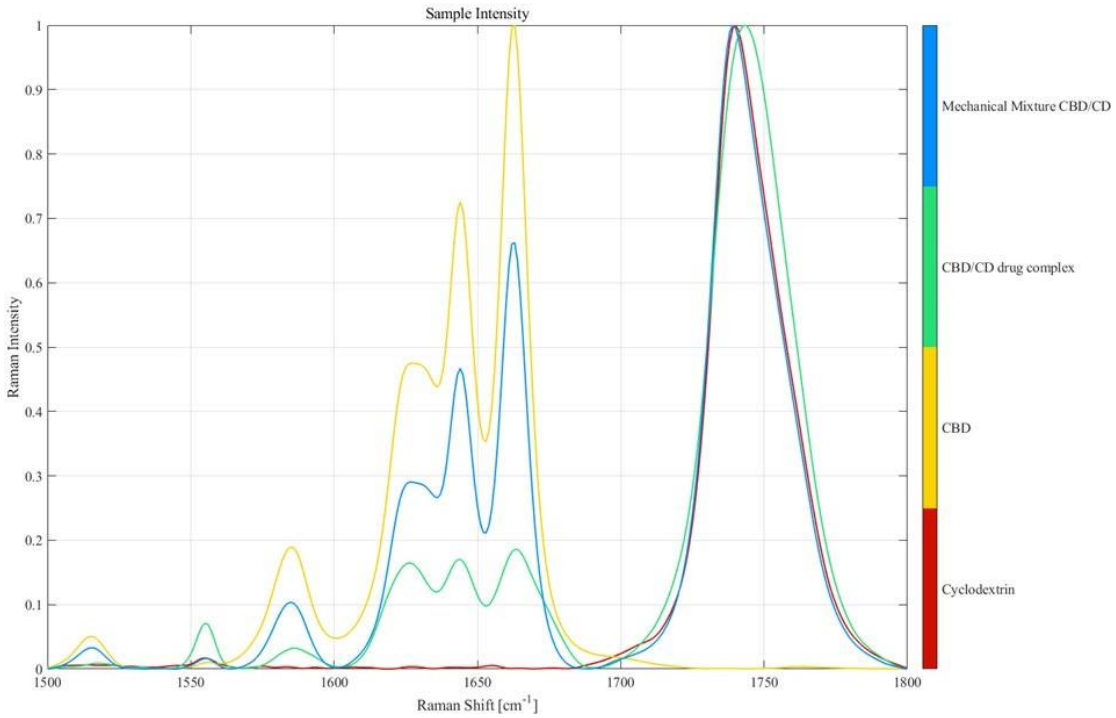

Figure 3*: Raman measurements of CBD (yellow), CD (red), mechanical mixture of CBD/CD (blue), and mixture of CBD/CD by the supercritical CO_2 inclusion process (green). The spectral range is from 1500-1800 cm^{-1} with applied rubber band baseline correction, 15 pt Savitzky-Golay smoothing, and peak maximum normalization.*

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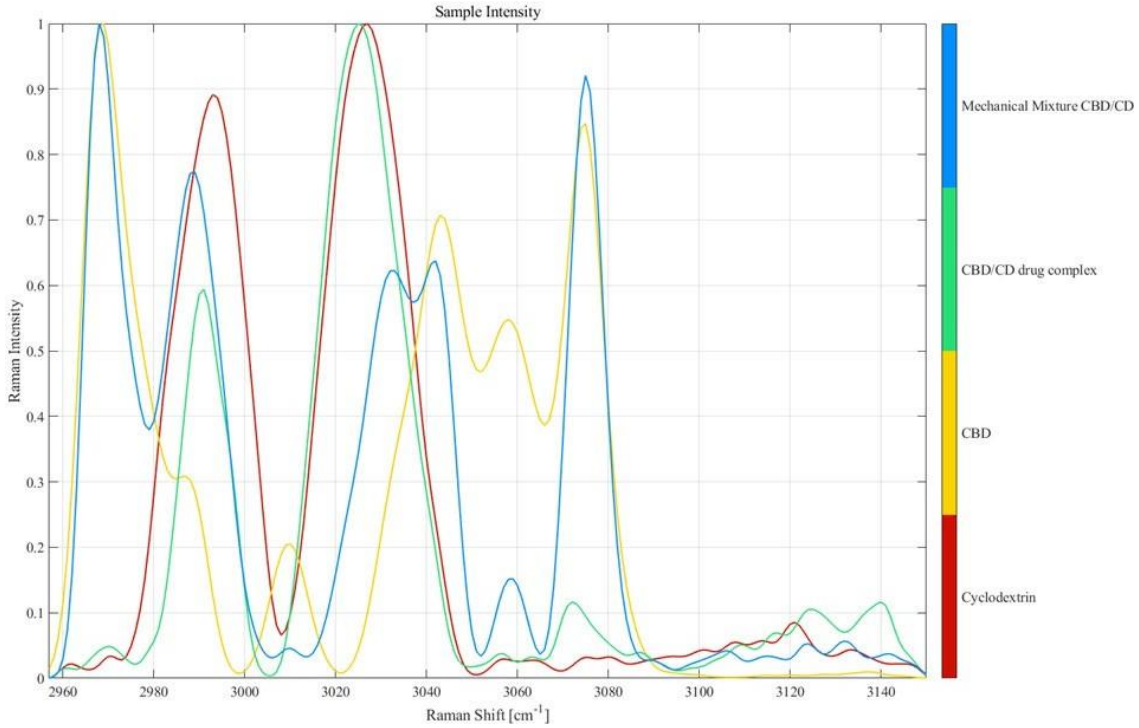

***Figure 4**: Raman measurements of CBD (yellow), CD (red), mechanical mixture of CBD/CD (blue), and mixture of CBD/CD by the supercritical CO_2 inclusion process (green). The spectral range is from 2956-3150 cm^{-1} with applied rubber band baseline correction, 15 pt Savitzky-Golay smoothing, and peak maximum normalization.*

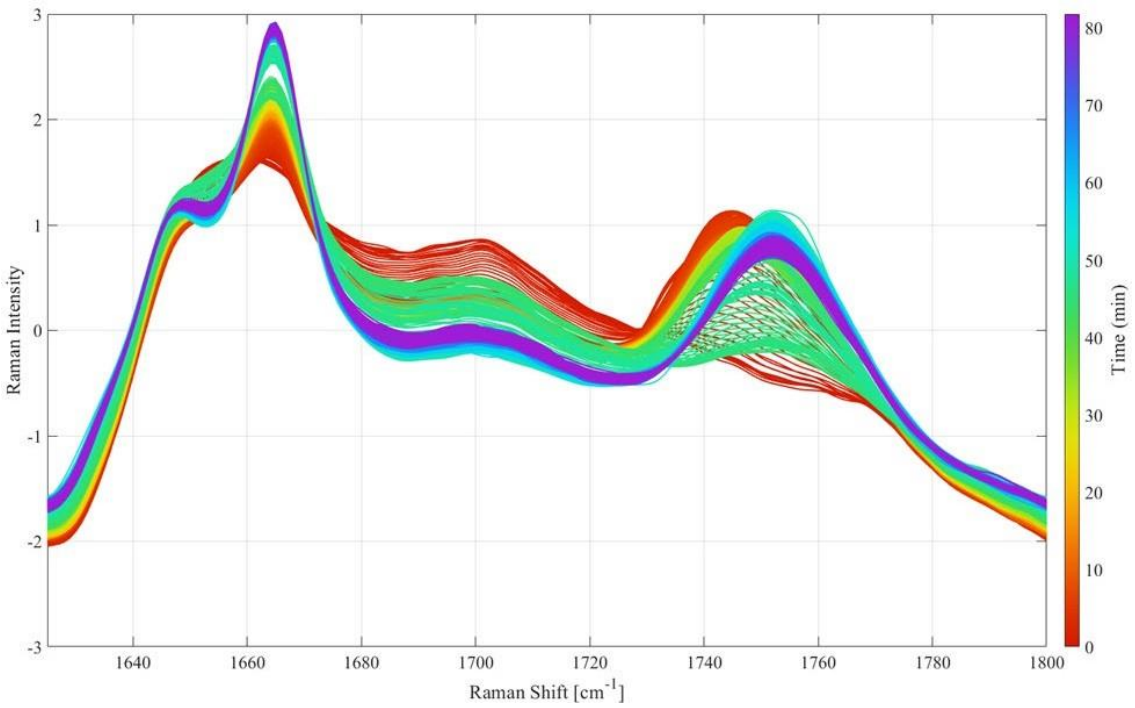

***Figure 5**: Raman in situ monitoring of the SC-CO_2 inclusion monitoring process. The spectral range is from 1625 cm^{-1} to 1800 cm^{-1} with applied rubber band baseline correction, 15 pt Savitzky-Golay smoothing, and SNV. Spectra were collected in 5s intervals for a total monitoring time of 82 minutes.*

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Having established the general Raman spectral behavior of the mechanical and supercritical mixture materials, we sought to monitor the supercritical inclusion *in situ* with the HyperFlux™ PRO Plus. Figure 5 shows the spectra captured over the 82 minutes of supercritical CO_2 mixing of a 1:1 molar ratio (200 mg to 1200 mg) of CBD to CD. Throughout the course of the process (red: beginning, purple: end) one can see a distinct shift of the carbonyl band. During initial solubilization of the CBD and CD the carbonyl band shifted from 1740 cm^{-1} to 1742 cm^{-1}. As the experiment progressed, the carbonyl band decreased and then re-appeared with a large shift to 1750 cm^{-1}. An interpretation of these spectral changes could be that the movement of the carbonyl from 1740 cm^{-1} to 1742 cm^{-1} represents the solubilization of the CD into the supercritical CO_2, and the larger shift towards 1750 cm^{-1} likely reflects the inclusion of CBD into the core of the CD ring.

The *in situ* spectra were further analyzed by MCR-ALS to deconvolve the time-series spectra into representative pure components to allow determination of the relative concentration of those pure components over time. The pure component spectra shown in Figure 6 represent the three components determined from the MCR-ALS analysis. The third component (blue) may be interpreted as starting CBD/CD mixture, as the cyclodextrin carbonyl band is centered at 1744 cm^{-1} and has some small amount of CBD aromatic bands from 1620 to 1680 cm^{-1}. The relative ratios of these aromatic bands will not necessarily reflect the solid sample spectra, as a bandpass filter was employed to block the interference from the CO_2. This component starts as one of the highest scores and decreases over time, which likely represents a non-included/non-solubilized CD/CBD mixture. The second component (orange) can be interpreted to represent the background spectrum of CO_2/scattering within the vessel. The first component (grey) is composed of three main peaks, the C=C alkene stretch from CBD which has slightly shifted to 1648 cm^{-1}, C=C ring stretch from CBD slightly shifted to 1665 cm^{-1}, and the carbonyl band from the modified cyclodextrin shifted to 1754 cm^{-1}. This large spectral shift reflects the Raman band changes seen between the end products of the mechanically mixed and supercritical inclusion CBD/CD samples. The band from the *in situ* study has a much larger shift (~10 cm^{-1}) than the 5 cm^{-1} shift seen in the recovered final product. This is likely a difference related to the fact that this measurement was done in supercritical CO_2 solvent which would be expected to manifest an additional shift in the C=O stretching band.

The trend plot of the MCR-ALS component scores is depicted in Figure 7. Component 1 continually decreases from the start of the mixing until the 45-minute mixing time is reached. At this point, large transitional changes are seen in the spectra until 47 minutes, at which point the first component decreases greatly in score. Component 3 steadily increases over time, remains consistent between the high variation regime of 45 to 47 minutes, and then quickly jumps to its near maximum score after 49 minutes of mixing time. If this score is correctly interpreted as the final CBD/CD inclusion complex that was recovered after the run, then it appears that the final phase of the inclusion takes place after the 49-minute mark. It is interesting because the data may suggest a two-step process, one that is gradual until a critical point is reached followed by a relatively rapid phase that largely remains steady (possibly in an equilibrium state) for the remainder of the time in which this process was monitored. It is possible that these steps may simply be related to the effects of the solubility kinetics. However, the spectral shifts are consistent with the effects previously shown with the inclusion of the CBD in CD. Additional studies would need to be performed to prove that the system can identify the timepoint of maximum inclusion *in situ*. However, this analysis proves that the Tornado HFPP can deliver the data acquisition speed, sensitivity, and chemical specificity that would be needed to elucidate the key chemical changes and kinetics of the inclusion of insoluble drugs into cyclodextrin in real-time.

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1.416.361.3444

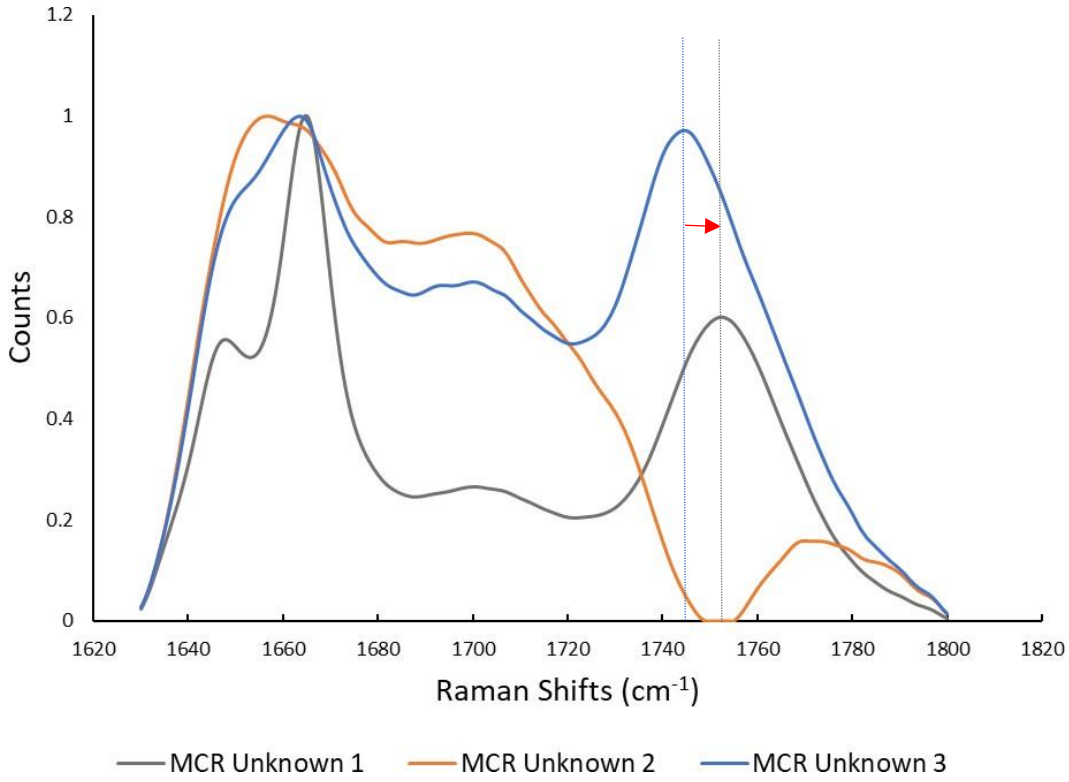

***Figure 6**: Pure component spectra resulting from the MCR-ALS analysis of the in situ monitoring of the supercritical CO_2 CBD/CD inclusion method.*

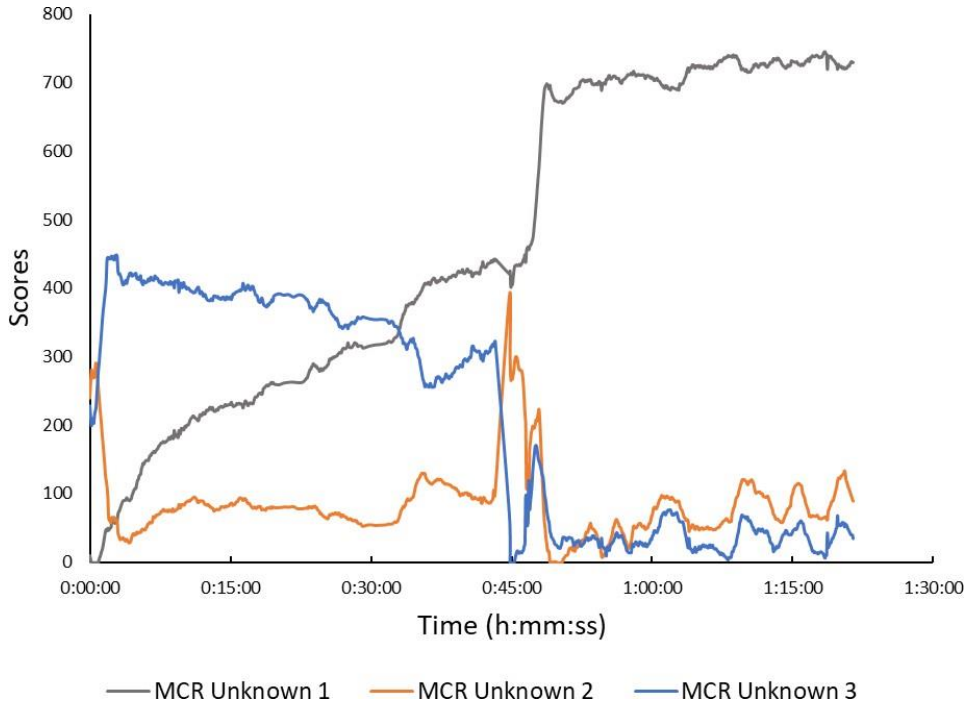

***Figure 7:** Pure component spectra scores from the MCR-ALS analysis of the in situ monitoring of the supercritical CO_2 CBD/CD inclusion method. The scores are plotted on the y-axis and the x-axis is process time.*

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CONCLUSION

The data presented in this paper indicate that HTVS-enabled Raman can be used to monitor a drug inclusion process. The spectral data clearly show the progression of the inclusion phenomenon in real time. This suggests that the Tornado Raman instrument can be used to follow this process, to verify that it occurs properly and perhaps to also verify that the maximum yield of the inclusion complex is obtained. This provides benefits during development in terms of process understanding but also suggests that Raman could be a valuable tool in monitoring a process such as this in a production scenario.

SOLUTIONS SUMMARY

- Tornado Raman Spectrometers allow for fast measurements with a high degree of chemical specificity that allows interpretation of process interactions at the molecular level.
- Tornado Raman Spectrometers can be used in development to establish understanding of drug/drug carrier interactions and monitor these interactions during the production process.
- Tornado Raman Spectrometers can be used to control the drug delivery system manufacturing process, leading to increased yields, greater process efficiency, and better outcomes for patients.

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