APPLICATION NOTE

BaySpec

Pervasive Spectroscopy

Characterization and Transformation Monitoring of Polymorphs of D-Mannitol with Raman Spectroscopy

Process monitoring with 1064nm Raman

Background

Polymorphs are different crystalline forms of the same pure substance in which molecules have different arrangements and/or different molecular conformation. Polymorphism is crucial in the pharmaceutical and fine chemical industries because different polymorphs have different physical and chemical properties, such as chemical reactivity, solubility, stability and dissolution rate. After all, the different properties of polymorphs would affect the bioavailability and storage of the active pharmaceutical ingredient (API) in the medicine. Due to the importance of polymorphism, screening of polymorphs and monitoring polymorphic transformation become necessary for research and development as well as quality assurance.

D-mannitol is a typical polymorphic crystalline solid which has been widely used in the pharmaceutical and food industries. Widespread use of D-mannitol occurs in the pharmaceutical industry as an excipient in final product by freeze-drying. Three major polymorphs of D-mannitol were reported: α-, β- and δ-, and one conventional method for identification of different polymorphs is X-ray which is very expensive and sensitive to the sample preparation.

Raman Spectroscopy is an ideal technique for this application with its several advantages: first, as a vibrational spectroscopy technique, Raman has the ability to distinguish the slight differences in molecular geometry; second, Raman analysis usually requires no sample preparation and is non-destructive method; third, the focus laser spot makes it can analysis with small amount of sample; last but not the least, the data acquisition of Raman spectrum is usually fast as in seconds. Beside of the above advantages from Raman technique, BaySpec's Raman spectrometers and microscopes provide high sensitivity, speed, flexibility and versatility as well as mature software interface for qualification, quantification and distribution analysis.

Methods

The δ form of D-mannitol is commercial available and was purchased from Sigma-Aldrich. The α polymorph was obtained through an antisolvent precipitation with acetone and distilled water.[1] The β polymorph was prepared from melt crystallization by heating the δ D-mannitol to 170 ℃ then allowing to crystallize through a natural cooling profile to room temperature.[2]

The Raman spectra of different polymorphs of D-mannitol were acquired with a BaySpec's RamSpec™-HR High-Resolution 1064nm Raman Spectrometer as in **Figure 1**, which provides turn-key solutions designed for best-in-class performance and resolution for dispersive 1064 nm Raman spectroscopy. BaySpec's unique 1064nm Raman Spectrometers dramatically expand sampling capabilities as they not only work for non-fluorescent samples such as mannitol, but also for fluorescent samples whose Raman spectra cannot be measured by shorter wavelengths.

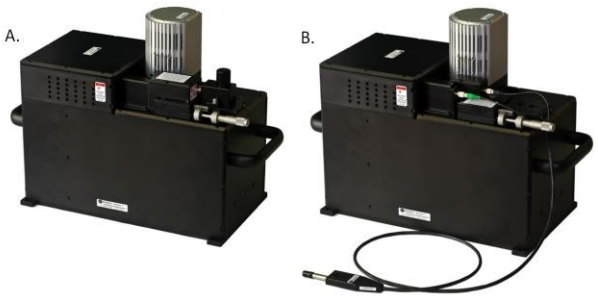

Figure 1: *BaySpec's RamSpec™-HR High-Resolution 1064nm Raman Spectrometer. Two exchangeable sample interfaces are provided: (A) direct sampling for higher optical efficiency and (B) fiber probe for flexibility.*

Results and Discussion

Raman spectroscopy can be successfully used to identify the polymorphs of D-mannitol. As the 1064nm Raman spectra of three polymorphs (α-, β- and δ-) shown in **Figure 2**, the differences between the three polymorphs is apparent. Not only the differences of

center positions of some peaks, but also the broadness and shape of some peaks of the Raman features around the Raman shift of 480, 885, 1125 cm^{-1}(**Figure 2A**) and between 2900 to 3000 cm^{-1}(**Figure 2B**) can be utilized to distinguish different polymorphs.

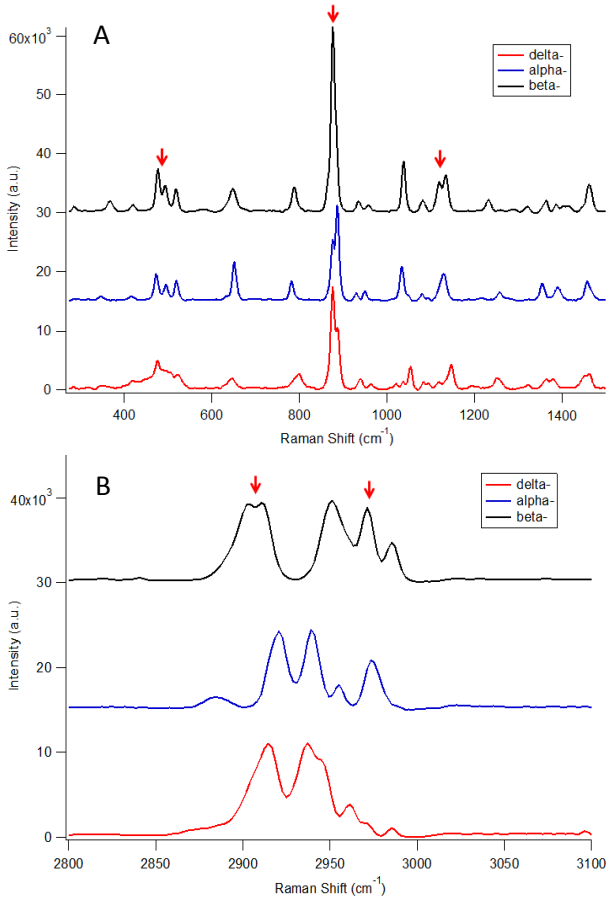

Figure 2: *1064nm Raman Spectra of Three different polymorphs of D-mannitol.*

Comparing the Raman features of three polymorphs in **Figure 2**, the α and β forms show more similarity and the major differences is located at 380, 885 and 1125 cm^{-1}. Meanwhile, the δ polymorph shows more unique Raman peaks which makes it easier to be distinguished from the other two forms. Thus, the identification of these three different polymorphs requires relatively high resolution of the Raman spectra collected. As an alternative for bulky, slow and expensive FT-Raman system, the RamSpec™-HR 1064nm Raman spectrometer can cover full spectral range (100-3200 cm^{-1}) as well as reach high resolution up to 4 cm^{-1} for providing more detail in the Raman spectrum for further analysis.

One of the major advantages for Raman technology comparing with other analytical methods is non-destructive and fast measurement which makes it very suitable for process monitoring. During the polymorphic transformation from δ form to β polymorph, Raman spectra of the sample were acquired every two minutes to monitoring the polymorphic transformation through the procedure of natural cooling. **Figure 3** shows the time-resolved *in situ* Raman spectral intensity change at spectral range of 1020~1180 cm^{-1}. Through this range, the Raman features of δ form centered at 1054 and 1146 cm^{-1} decreased with the time while the Raman features of β polymorph centered at 1038, 1118 and 1137 increased. Moreover, the δ form D-mannitoal had been mostly changed to β form at the time of 36 minutes.

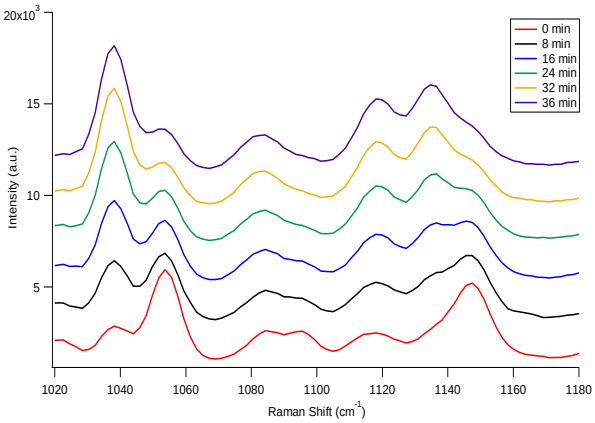

Figure 3: *Raman spectra of D-mannitol during the polymorph transformation from δ form to β polymorph.*

Figure 4 shows the correlations between the Raman intensities of peak centered at 1038 and 1054 cm-1 and the time. As the representative Raman peak of δ form D-mannitoal at 1054 cm^{-1}, the Raman intensity gradually decreased with the time through the naturally cooling procedure. In contrast, the increasing intensity at the Raman peak of 1038 cm^{-1} indicates the forming of the β polymorph. The simultaneous peak intensity variance demonstrated the ability of process monitoring during the polymorphic transformation with 1064nm Raman spectrometer.

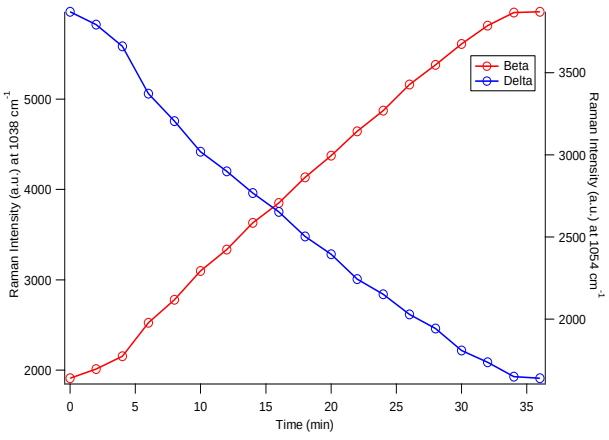

Figure 4: *in situ Raman intensity variances at Raman peaks centered at 1308 (Red, from β polymorph) and 1054 cm^{-1}(Blue, from δ polymorph)*

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After all, 1064nm Raman imaging of the mixture sample of α and β form of D-mannitol, which has most similarity on the Raman features between these three polymorphs, was executed within an area of 800 x 700 µm (80*70 pixels, bright-view image was shown in **Figure 5B**). BaySpec's Nomadic™ Raman multi-wavelength microscope was utilized for this application. As the unique Raman features (**Figure 5A**) for each polymorph were selected by markers with different colors to reconstruct the Raman image. The color-coded Raman image, shown in **Figure 5C,** successfully indicated the distributions of these two polymorphs in the scanned region.

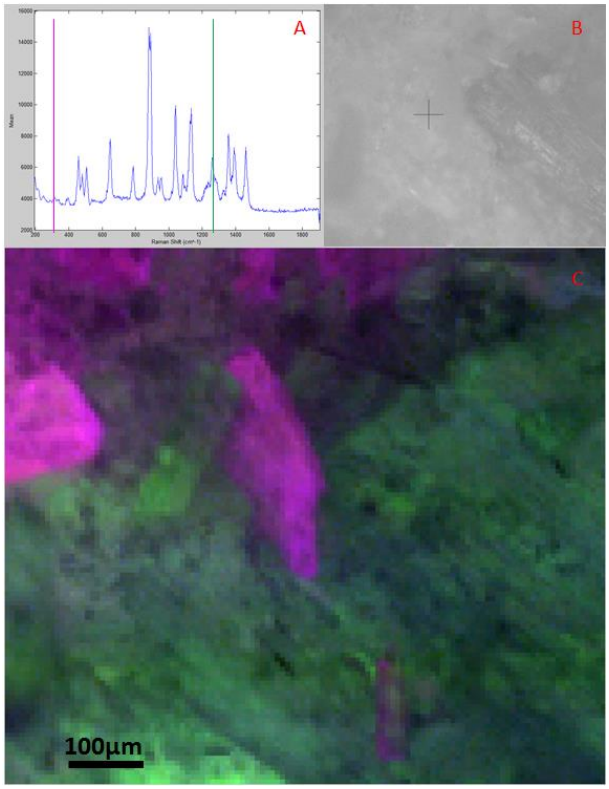

Figure 5: *Color-coded Raman imaging of the mixture sample of α- and β- forms of D-mannitol (C). (A) indicates the selected unique Raman features of different chemical constituents for Raman imaging and (B) is the bright-view image of scanning area.*

Conclusion

Based on these experiments, 1064nm dispersive Raman is demonstrated as a viable new option and non-destructive method to identify polymorph and apply process monitoring. With the high resolution Raman spectra provided, the differences of the Raman features from polymorphs can be easily utilized for the purpose of polymorph identification, process monitoring and distribution characterization. Equipped with variance measurement accessories, 96-well micro-plate Raman reader (**Figure 6**), flowing cell sampling interface and *in situ* monitoring stage as examples, fully automatic Raman spectra measurement for identification and process monitoring can be easily applied for R&D or quality assurance purpose. Further, coupling with chemometrics tools such as principal component analysis (PCA) and partial least squares regression (PLS), a viable new option and non-destructive method of identification and quantification of the chemical compositions automatically can be achieved. Moreover, the instrument and methodology can be easily adapted to many other R&D areas in pharmaceutical industry.

Figure 6: *96-well micro-plate sampling accessory for automatic Raman acquisition. Inset: software interface during an automated measurement with Identification Mode on.*

References

1) Burger, A., et al., *J. Pharm. Sci.*, **2000**, 89(4), 457-468.
2) Bruni, G., et al., *J. Therm. Anal. Calorim.*, **2009**, 95, 871-876.

(A) Fiber optical probe for bioreactor monitoring. Standard PG 13.5 connector and simple maintenance.
(B) High temperature fiber probe for process monitoring. Pressure sleeve specified to 3000 psi and up to 600 ℃.
(C) Optional IP66 NEMA enclosure provides instrument protection in outdoor and industrial environments.
(D) Reaction monitoring set-up with BaySpec's RamSpec Benchtop Raman spectrometer and high temperature probe.

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