In-line monitoring of a pharmaceutical freeze-drying process

Benefits at a glance
- Non-destructive, non-contact analysis of a lyophilization process
- Spectroscopic identification of various solid forms of a pharmaceutical compound
- Real-time process understanding and control

Introduction

The purpose of this study was to evaluate Raman spectroscopy as a PAT tool for in-line monitoring of a pharmaceutical freeze-drying process. Also known as lyophilization, freeze-drying is a low temperature drying process used to turn solutions of heat-labile materials into stable, lightweight solids for lower cost transportation and storage. The first step in the process is freezing, turning the liquid water to ice and the solutes to a crystallized or amorphous solid. The second step is a primary drying process in which the ice is removed by sublimation under vacuum. Finally, a secondary drying step under deep vacuum is used to remove most of the unfrozen water by desorption.

In this application, a Raman analyzer with a non-contact optical probe was used to monitor the freeze-drying of a 5% w/v solution of D-mannitol non-destructively and without interfering with the process itself. Rich data were acquired that could be used to automate control of the process based on real-time feedback and predetermined spectral set points.

Experimental

A Raman analyzer was used in combination with a non-contact fiber optic probe to non-invasively monitor the process in real-time. Interfacing with the sample was uncomplicated, with the probe simply secured inside the freeze drier immediately above the product (Figure 1). Laser excitation was provided by a 785 nm NIR diode laser, and all spectra were recorded with an exposure time of 30 seconds at a resolution of 4 cm^{-1}. Data collection, data transfer, and data analysis by principal components analysis were automated, and spectra were collected every minute. Over 1500 spectra were collected during the approximately 25 hour duration of the process.

The various mannitol states can be easily distinguished by the differences in their Raman spectra between 1000 and 1170 cm^{-1}, so this spectral region was monitored throughout the freeze-drying process. Since ice has a Raman band at 215 cm^{-1}, the intensity of this band was monitored during the freezing and primary drying steps. Liquid water and sodium chloride produce either no Raman signal in the region of interest or do not produce a Raman all. Therefore, all visible Raman bands in the collected spectra, other than the ice band, can be determined to have originated from mannitol.

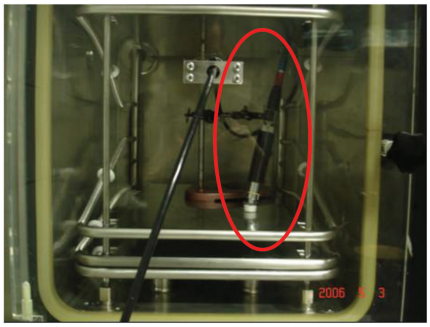

Figure 1: Experimental configuration for real-time in-line monitoring of pharmaceutical freeze-drying using a non-contact Raman probe. The Raman probe is oriented above the product on the freeze-drier tray. Adapted with permission from Ref. 1 (supporting information). © 2007 American Chemical Society.

Results

Figure 2 shows a plot of the principal component (PC) scores for each spectrum in the experiment. The loading vectors for PC 1 and PC 2 bear spectral characteristics of crystallized mannitol and ice, respectively. The fact that these two loading vectors correspond to uncorrelated phenomena occurring over the course of the process indicates further that they are, in fact, associated with crystallization of mannitol and formation of ice. This indicates that they can be used as spectral markers for these events.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

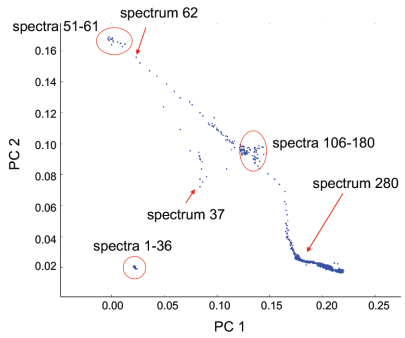

Figure 2: PCA scores plot for Raman spectra collected during the freeze-drying process. PC 1 corresponds to mannitol crystallization, and PC 2 corresponds to ice formation. (Reprinted with permission from Ref. 1. © 2007 American Chemical Society.)

In the first 25 minutes of the process, the mannitol solution was cooled and brought to equilibrium below its freezing point of −5 °C but above −10 °C, where ice nucleation starts. This procedure helps ensure that the ice crystals form homogeneously throughout the vial. Spectra 1 to 25 correspond to pure aqueous mannitol solution with little or no crystallized mannitol or ice, as indicated by the low scores for both PC 1 and PC 2 in Figure 2.

After 25 minutes, the temperature of the system was decreased from −5 °C to −45 °C at 2 °C/minute. The conversion of liquid water to ice started after 37 minutes, which is made evident by the increase in PC 2 scores after this point in Figure 2. Between 51 and 61 minutes, the scores form a new cluster in the upper left-hand region of the plot—corresponding to low values for PC 1 and high values for PC 2—indicating that the liquid water has frozen to ice but that no mannitol crystals have formed yet.

From 62 to 106 minutes, the scores form a linear progression in Figure 2, from the upper-left toward the lower-right regions of the plot, indicating a decrease in PC 2 (ice) and increase in PC 1 (crystallized mannitol). At the end of the mannitol crystallization, spectra 106 to 175 remained constant and are clustered in the plot, showing that no solid-state conversions occurred during this portion of the process.

After 175 minutes, the shelf temperature was increased to −15 °C and the pressure reduced to 1 mbar in order to initiate the primary drying step. Once this began, the PC 2 scores from the spectra decreased again between 180 and 280 minutes.

During the freezing step, mannitol crystallized mainly as mannitol hemihydrate, an undesirable form because of possible water release during storage. Therefore, a secondary drying step was performed using a shelf temperature of 40 °C. Under these conditions, Raman spectroscopy was able to monitor the conversion of mannitol hemihydrate to α-mannitol (Figure 3).

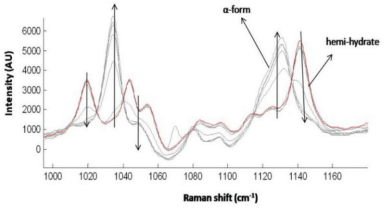

Figure 3: Mannitol solid-state changes during secondary drying. Adapted with permission from Ref. 1 (supporting information). © 2007 American Chemical Society.

Conclusions

Raman spectroscopy was found to be an ideal PAT tool for in-line, non-invasive real-time analysis of a freeze-drying process. Raman was able to monitor both the evolution of the product's solid form and several of the phenomena occurring throughout the process: the onset of water to ice conversion, end of ice crystallization, onset and end of mannitol crystallization, and mannitol solid-state conversion. Although automated control was not specifically tested in this study, these results demonstrate that the speed and quality of Raman data supports real-time, automated control of manufacturing processes.

References

1. De Beer, T R. M. et al. "Implementation of a Process Analytical Technology System in a Freeze-Drying Process Using Raman Spectroscopy for In-Line Process Monitoring." *Anal. Chem.*, **Nov. 1, 2007**, 7992–8003.