

NIR was recently used as an in-line process analyzer for monitoring protein unfolding and protein–lyoprotectant interactions during drying [21]. The amide A/II band near 4850 cm^{-1} was monitored along with the water absorbance band near 5160 cm^{-1} . This amide A/II frequency was found to correlate well with the water absorbance intensity during protein dehydration in the absence of protein unfolding, whereas deviation from the linear relationship was found to be related to protein unfolding. For sucrose formulations, the amide A/II frequencies decrease immediately after sublimation, suggesting an increase in protein–sucrose hydrogen bond interaction. This approach could provide more insights about protein conformational stability and the lyoprotectant interaction with protein at real time during the dehydration process.

Even though NIR has demonstrated some utility for in-line process monitoring, the technique has several limitations. First, NIR is based on the detection of a single vial in the edge, and only small part of the cake is measured by the NIR probe. Therefore, the result measured is not representative of the entire batch. Second, this technique requires an unobstructed view of the NIR probe to the contents of the vial and the insertion of a fiber-optic cable into the chamber, which makes it difficult to integrate into a manufacturing scale lyophilizer. While NIR has low potential as an in-line PAT tool for freeze-drying, it could find off-line applications as a nondestructive method for high throughput residual water measurements.

Raman Spectroscopy

Since water and ice are weak Raman scatterers, Raman spectroscopy is becoming popular for monitoring formulation characteristics during the freeze-drying process. De Beer et al. have studied Raman as the PAT to monitor the endpoint of primary drying. The Raman probe was placed above the product vial in the drying chamber. The Raman technique gave a significantly lower endpoint of primary drying, which was due to the inappropriate placement of Raman probe [3]. However, Raman spectroscopy was able to provide information about the endpoint of freezing and the mannitol solid-state conversion throughout the entire process. It was reported that a combination of Raman and Lyotrack allows the monitoring of nearly all process aspects. Raman spectroscopy can provide insights into the product behavior during freezing stage and also the dynamics of bound water removal during secondary drying. Conversely, Lyotrack gives information about the primary drying endpoint [4].

Recently, Raman spectroscopy was used for in-line monitoring of a microscale freeze-drying process [8]. The effect of cooling rate and annealing step on the solid-state formation of mannitol was studied. Principal component analysis was used to qualitatively analyze the solid-state forms of mannitol, while classical least-squares regression analysis was used to estimate the solid-state form ratios of each polymorphism. The results showed that mannitol hemihydrate emerged and subsequently transformed to more stable forms during the secondary drying step. Similar to NIR, the Raman measurement is based on one vial, and it is not representative of