

Off-Line Determination of Residual Water in Freeze-Dried Solids

Water exhibits strong absorption in the NIR region around 970, 1190, 1450, and 1940 nm [36]. Many efforts have been made to evaluate NIR spectroscopy as a fast nondestructive approach to determine the residual moisture content in the lyophilized products. Residual water was determined in freeze-dried sucrose samples using NIR diffuse reflectance spectrometry by scanning through the bottom of the glass vial [7]. A good correlation ($r^2=0.97$) was observed between residual water determined by traditional Karl Fischer method and NIR. The impacts of cake porosity, buffer concentration, and surfactant on NIR signal were also studied for a lyophilized monoclonal antibody system [14]. These factors do not have significant effect on the residual moisture when the cake thickness and diameter was much larger than the NIR penetration depth. However, it was found that the disaccharide concentration has a large impact on the NIR determined moisture content, which results from the strong NIR absorbance of disaccharide at the same wavelength as residual water. Therefore, the NIR method for moisture quantification is highly sensitive to the drug formulation, and a calibration curve needs to be developed against the standard Karl Fischer method. It was reported that the calibration curve developed for one formulation cannot be used for another drug concentration [13]. Even for a given formulation, samples from different batches could give differences in the correlation between NIR and Karl Fischer. Therefore, it is critical to develop robust calibration curves incorporating all relevant factors and manufacturing process variables.

In-Line Process Monitoring for Protein Conformation and Protein–Excipient Interactions

NIR has also been used as in-line PAT tool to monitor the lyophilization process since water and ice give strong signals in NIR spectra. De Beer et al. have studied the performance of a NIR probe directing to the sidewall of a vial for mannitol formulation [4]. Chemometric tools were used to extract useful information from the large raw data sets, and it was found that mannitol starts to crystallize when ice crystallization is complete. Since sublimation finishes at the bottom center of the vial, the drying endpoint can also be measured by the NIR tool. However, NIR may underestimate the drying endpoint as compared to the endpoint measured by Lyotrap method.

Another application of NIR spectroscopy is the differentiation of mannitol hydrate and surface water. The different crystalline mannitol polymorphs can be distinguished via NIR in the 4330–4450 cm^{-1} spectral range, and a nondestructive method was developed to determine the amount of metastable mannitol hydrate and surface water in lyophilized products [3]. This study indicated that NIR could be employed to monitor the formation of mannitol hydrate during lyophilization process.