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Near-Infrared Analysis of Critical Parameters in Lyophilized Materials

Jeffrey Hirsch, Thermo Electron Corporation, Madison, WI, USA

Key Words

- Nicolet Antaris
- FT-NIR
- Lyophilization
- Moisture
- Thrombin

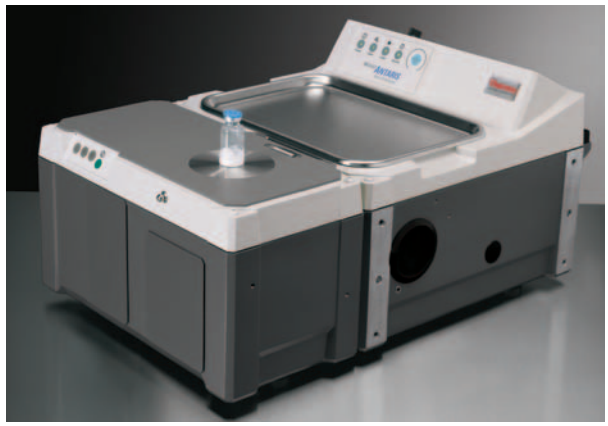
Abstract

Lyophilized materials are challenging samples for QA/QC measurement due to the inability to open the container without corrupting the product. Near-infrared analysis presents itself as the QC method of choice for lyophilized materials due to its ability to scan through containers like glass or plastic to analyze the sample inside non-destructively. The current study demonstrates the performance of the Nicolet™ Antaris™ Fourier Transform Near-Infrared (FT-NIR) spectrometer in analyzing lyophilized samples of thrombin, a topical coagulant commonly used in the medical and dental fields. Key stability parameters for lyophilized thrombin are moisture and potency which can be predicted simultaneously from a single spectrum using multivariate analysis. Other considerations for the analysis of lyophilized cakes are also discussed.

Introduction

Lyophilization is a common process in both the food and pharmaceutical industries that negates the need for sample refrigeration while dramatically increasing shelf life. Products that would normally spoil after a few months of refrigeration can be rendered stable, in some cases, for years at room temperature. Lyophilization works by removing residual moisture in a sample through sublimation (the process of transitioning water from the solid phase to the vapor phase without going through the liquid phase). Sublimation is the process at the heart of any lyophilization. If one was to attempt to remove water from a sample simply by heating to send the water into the vapor phase, the sample would be destroyed.

The process of sublimation begins with a solution of target compound along with various buffers and bulking agents injected into a serum vial. The vial is then partially stoppered and the solution is frozen below its glass temperature (T_g) or, in the case of crystalline compounds, its Eutectic temperature. Glassing temperature is the temperature below which the material is essentially solid so removal of water can proceed efficiently. The pressure is then reduced and the freeze drying process begins with the primary drying stage. After the bulk moisture is removed in the primary drying phase, the residual moisture (sometimes still as great as 8% by weight) is removed in the secondary drying phase. Here, the temperature is slowly increased as the pressure is reduced further until the desired degree of dryness is achieved. The serum vials are then stoppered and sealed.



Nicolet Antaris FT-NIR analyzer

The most fundamentally challenging issue with lyophilized materials is how to analyze them once they are sealed. Fine chemicals or proteins that are lyophilized have many chemical and physical properties associated with them but without proper analytical techniques, there is no way to measure them to be sure that the product will be safe and effective. Currently, lyophilized materials are analyzed by batch sampling where a small number of samples are pulled from a lot, opened, and analyzed for parameters such as moisture, concentration of API, or efficacy. Batch testing of lyophilized materials is ineffective for several reasons: sample subsets are never guaranteed to be representative of the whole lot; the samples are destroyed; and protocols like titrations, PAGE or ELISA are laborious, complicated, and expensive.

FT-NIR spectroscopy is a modern, easy-to-use technique that uses absorbing vibrations of molecules. FT-NIR analysis allows the user to scan through packing materials like polyethylene bags or glass vials to gain information about the sample within. In the case of lyophilized materials, low-energy light can readily penetrate through a serum vial to reach the analyte of interest without damaging it. Once the spectral information is collected, multivariate analysis techniques can retrieve information about multiple chemical or physical parameters all from the same single spectrum. Typical scan times for a lyophilized sample by FT-NIR are 15 to 30 seconds which, when compared with the 30-60 minutes necessary for a Karl Fischer titration, is extremely efficient.

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The current study details the analysis of a lyophilized protein product, thrombin, a topical coagulant, by FT-NIR. The two critical parameters for thrombin are moisture (usually below 1.0%) and potency. Traditionally, the moisture measurements are carried out by Karl Fischer titration, a USP-referenced technique (USP <921>). Potency is established using light scattering measurements after the sample is titrated with plasma. If the sample potency is high, then the thrombin will coagulate the plasma creating particulates. The greater the potency, the more particulates are created, and the greater the light scattered from the sample.
Both of the techniques described above are destructive and require the use of a skilled operator. In addition, both are time consuming and use consumables such as solvents, plasma, or reagents.

Another part of this study details the spectroscopic differences between lyophilized materials that have their cakes completely intact and cakes that may have settled in shipping. This is an important distinction in that even though a product may be suitable for use, it may have experienced some settling which could interfere with a calibration that used only intact cake.

Experimental

Two sets of ten thrombin samples were used to establish calibrations for moisture and potency. The samples were pulled from finished product lots and analyzed through the vial using a Nicolet Antaris FT-NIR analyzer. The Autosampler RS attachment shown below was used to collect all the sample data without operator interaction. Thirty-two scans were taken of each sample and co-averaged at a resolution of 4.0 cm^{-1}. Each sample took approximately 20 seconds for analysis. The wavelength range scanned was 4000 cm^{-1} to 10000 cm^{-1}. Once the samples were analyzed on the FT-NIR they were measured destructively using the primary techniques – Karl Fischer titration for the ten moisture samples and light scattering plasma titration for potency. These reference numbers were then combined with the spectral data in multivariate analysis methods using TQ Analyst™ software, Thermo Electron's chemometric analysis package.

Autosampler RS accessory

Results and Discussion

Moisture

Spectral data from the Nicolet Antaris FT-NIR analyzer was combined with primary numbers from Karl Fischer titration for ten samples. The moisture values ranged approximately from 0.5% to 0.8%. These data were combined using TQ Analyst software to make a chemometric model using the SMLR (Stepwise Multiple Linear Regression) algorithm. Data pretreatment of Near IR spectra is common to help the various algorithms predict analytes accurately. In some cases, the first or second derivative of the raw spectrum may prove more effective for measurement. Other pretreatments include pathlength algorithms like Multiplicative Scatter Correction or Standard Normal Variate, smoothing, or baseline corrections. In this case, the pretreatments were minimal as moisture is usually a strong absorber in the Near IR region. The second derivative of the spectra was used for making the calibration curve with a 9,2 Norris smoothing filter. The regions chosen were constrained around 7000 cm^{-1}, the first water overtone band. The water overtone region for the ten standards is shown in Figure 1 where moisture concentration differences are easy to see from the raw spectra.

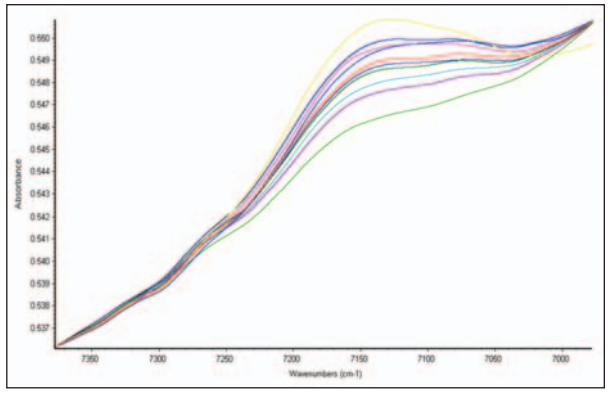

Figure 1: Water Overtone

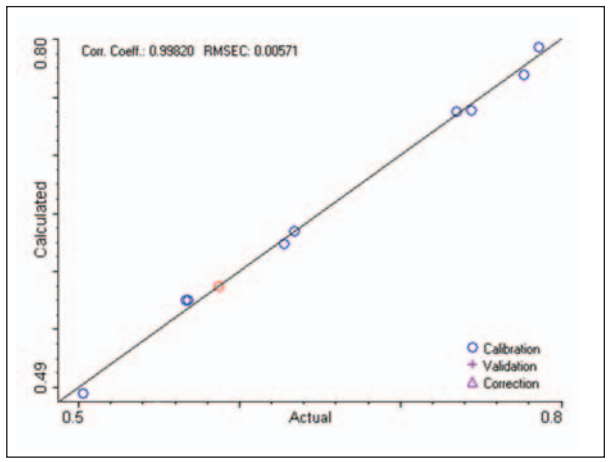

Figure 2: Moisture Calibration

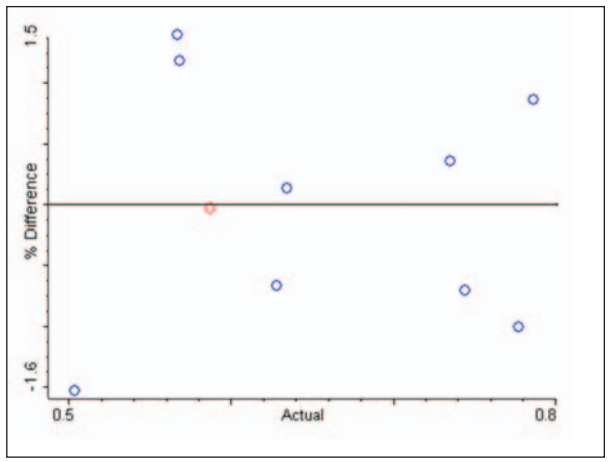

Figure 3: Moisture Residual

The calibration curve and % residual for moisture are shown in Figures 2 and 3. The correlation coefficient (how the data is fit by a straight line – 1.0 is theoretically perfect) is 0.998 with a Root Mean Square Error of Calibration (RMSEC) of 0.005. Both of these parameters describe an excellent fit to the data. Cross validation is a reliable way to gauge the stability of a certain method. One by one, each calibration standard is removed from a calibration and predicted against the remaining nine calibration standards. If the method is robust, the predicted values of these removed standards will not be very far from their original values. If the calibration seems to get significantly worse upon cross validation, then the method should be reviewed and perhaps have more standards added. Cross validation for moisture in thrombin gave a correlation coefficient of 0.984 with a Root Mean Square Error of Cross Validation (RMSECV) of 0.018. Although cross validation showed some change in the line fit from the calibration curve, the RMSECV was only a few times greater than the RMSEC, indicating a relatively stable method. The cross validation curve for moisture is shown in Figure 4.

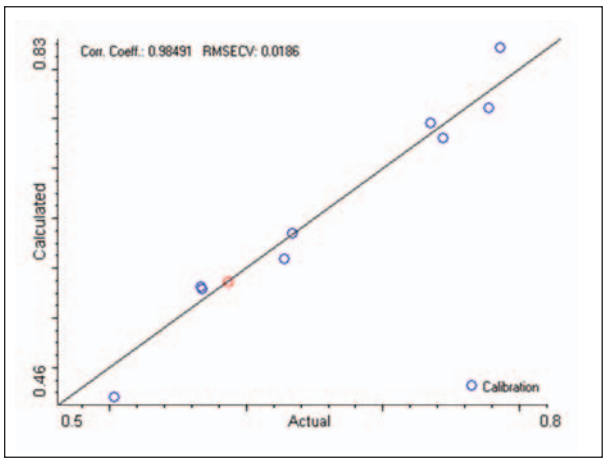

Figure 4: Cross validation of moisture

Because FT-NIR is a secondary technique, any error that is implicit in the primary method is, by definition, transferred to the FT-NIR calibration. As the error in the titration analysis can be minimized, the calibration curve for the NIR data should, in turn, improve even more.

Potency

Potency analysis was done by correlating reference potency data derived from titration scattering measurements with spectral data on a second set of ten thrombin samples. These samples had potency values from approximately 29,000 to 33,000.

The pretreatment for this data set was slightly different than that for moisture. Here we used the Partial Least Squares (PLS) algorithm on the second derivative spectra with a Norris 9, 5 smoothing filter. The pathlength compensation used was a Multiplicative Scatter Correction. The analysis region for potency was 6000 cm^{-1} to 6800 cm^{-1} which is in between, but not part of, the water resonances. The calibration curve in this case (Figure 5) was excellent with a correlation coefficient of 0.999 and an RMSEC of 21.9. The percent residual (Figure 6) showed prediction values for potency of ±0.09% although one sample had a residual value of -0.18. The Predicted Error Sum of Squares (PRESS) also showed a reasonable trend (Figure 7). For a reasonable calibration curve, the PRESS plot should start high, tend towards a minimum and then stay flat or increase slightly. Cross validation for this calibration showed the residual increase to approximately ±2.0%, however, the high and low standards had higher error than this due to the fact that they were under represented in the calibration.

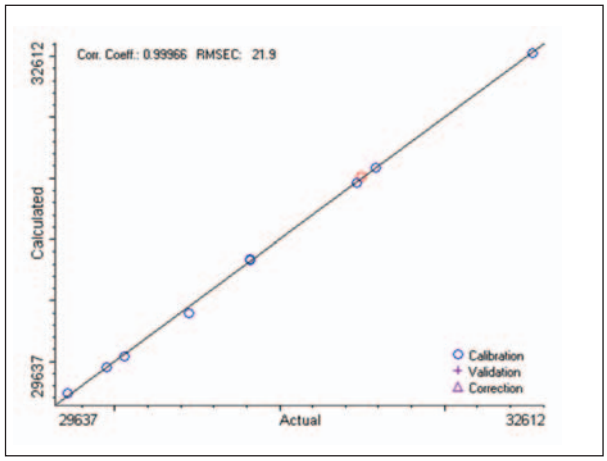

Figure 5: Potency Calibration

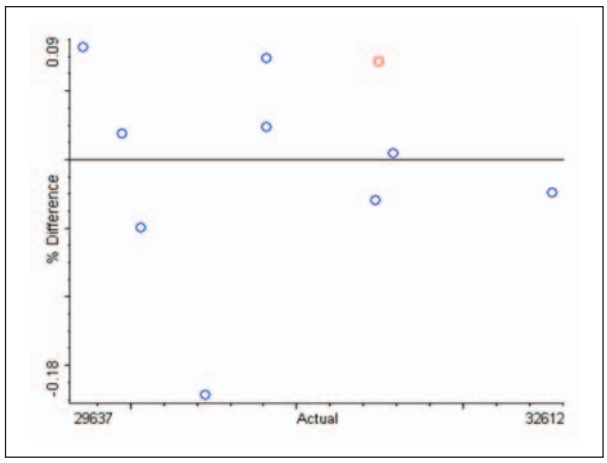

Figure 6: Potency Residual

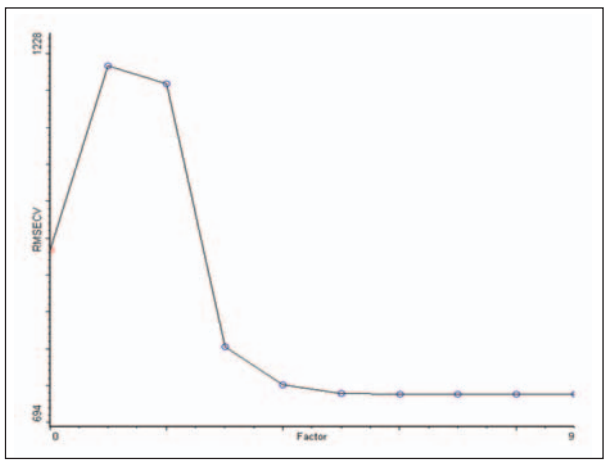

Figure 7: PRESS Plot for Potency

Cake Settling by Discriminant Analysis

A major concern with lyophilized materials is settling. Once a sample is put through a lyophilization cycle, it should emerge as an intact cake. After boxing and shipping, however, many samples can settle leaving a powdery morphology instead of the solid single cake. Can an intact cake be differentiated spectrally from a settled or powdery cake?

Using TQ Analyst software's Discriminant Analysis algorithm, we show that these two types of sample can be readily distinguished. Figure 8 shows a principal component plot of ten thrombin samples with intact cakes and the same ten samples after they were shaken into powder. Principal component analysis is a way of reducing spectral variability (multiple wavelengths and absorbances) to a useable amount of data, the principal components. When principal component data for samples are plotted on an x-y plane, it becomes very easy to distinguish between spectrally different populations. It is clear from the principal component plot (Figure 8) that cakes that have settled can be easily identified spectrally and, if necessary, compensated for. Calibrations using the settled samples instead of the intact cake samples gave similar results for the above quantifications.

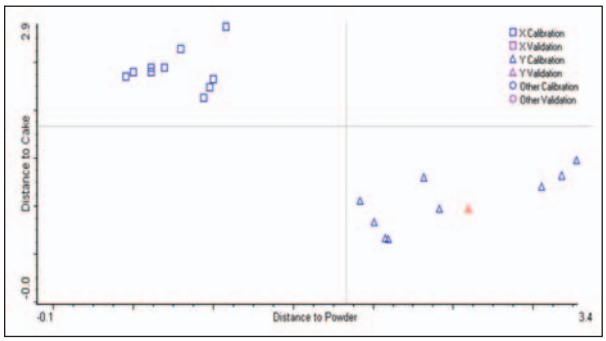

Figure 8: Principal component plot of settled vs. intact Lyo cakes

The cake settling data below shows that although there are true spectroscopic differences between the two sample sets (cakes and settled cakes) these may be manifested as simple scattering offsets. If one sample scatters more than another, then the two baselines will be different. There may also be absorbance differences due to more settled cakes. In this case, based on the data, variation from sample settling can be compensated for by adding a small number of standards that represent this type of variation. If your calibration was developed using intact cakes but data from settled cakes needs to be added without changing the calibration, then standard spectra from settled cakes can be added to the original calibration. This can readily compensate for sample settling effects.

Conclusion

FT-NIR analysis of lyophilized materials using the Nicolet Antaris FT-NIR analyzer was shown to be an effective, rapid, and non-destructive method for analyzing lyophilized materials. In this case, both moisture and potency were examined with the primary methods being Karl Fischer titration and colloidal light scattering, respectively. Also, it was shown that intact lyophilized material cakes can be distinguished from cakes that have settled using principal component analysis.

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Australia
+61 2 8844 9500
Austria
+43 1 333 50340
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