

Quantitative Characterization of Lactose Crystalline Forms: A Case Study to Illustrate the Utility of FT-NIR as a Tool for the Analysis of the Crystalline Composition of Solid Materials

M. S. Kemper and W. J. McCarthy
Thermo Nicolet Industrial Solutions, Madison, WI, USA

P. E. Luner, A. D. Patel and J. J. Seyer
University of Iowa, School of Pharmacy, Iowa City, IA, USA

Lactose is an important raw material used in many food and pharmaceutical products. Alpha, alpha monohydrate and amorphous lactose are among the most common forms of the material and were used for this study. The crystalline nature of the starting material can ultimately affect the production process and, hence, the quality of the final product. The Fourier transform near-infrared (FT-NIR) spectra of these three major forms are substantially different from one another making characterization relatively straightforward with the proper experimental design for calibration. This makes FT-NIR a potential candidate for use as a routine tool for this type of analysis. FT-NIR has the added benefits of being fast and easy to use. Measurements can be made to be essentially operator independent, and non-technical personnel can conduct standard testing in the receiving area or production area. The success of the FT-NIR method with lactose suggests that other materials can be similarly characterized.

KEY WORDS: Antaris, FT-NIR, Quantification,
Diffuse Reflectance, Lactose, Crystallinity

INTRODUCTION

The use of near-infrared (NIR) spectroscopy in the pharmaceutical industry has increased substantially over the past several years.¹⁻³ Among pharmaceutical scientists, raw material identification is one of the oldest and best known applications of the NIR technique.⁴ However, with the maturation of the Fourier transform NIR (FT-NIR) market, NIR is also becoming an important tool for assessing the fitness of materials for their intended use. Even though a material is identified as a specified chemical entity, the physical status of this material before and after processing can have a profound effect on final product quality and shelf life. Other physical parameters such as particle size will also be critical to trouble-free processing.

With the increased resolution provided by FT-NIR instrumentation compared to dispersive NIR equipment, the parameters that affect product or material quality can be assessed more rigorously because of the improved spectral specificity. This, in turn, makes aberrant spectral qualities related to the material or product quality easier to detect.

In this study, FT-NIR was used to quantitatively characterize binary and ternary mixtures of lactose, an important excipient used in many types of pharmaceutical dosage forms. The characterization of lactose is desirable because the ratio of the different crystalline forms in individual batches of material can have a profound effect on the performance of the final product in which it is used. Inevitably, that material will be adulterated with other lactose forms as contaminants. The negative consequences of using or producing inappropriate lactose could be manifested in the manufacturing process, resulting in product instability. Hence, a tool for routinely determining the amount of various forms of lactose in raw materials and final products would be quite useful. Historically, this analysis has been accomplished using techniques such as x-ray powder diffraction (XRPD), thermogravimetric analysis (TGA), proton nuclear magnetic resonance (p-NMR), differential scanning calorimetry (DSC), polarimetry and moisture sorption. None of these techniques are easy to use routinely, and some, such as

polarimetry and moisture sorption, do not detect all of the possible lactose forms.

This investigation serves as a case study to illustrate the utility of FT-NIR for the characterization of lactose. FT-NIR is well suited for the facile characterization of physical anomalies such as polymorphic constitution and deviations in the particle size and shape. FT-NIR is a fast technique that is easy to use and well suited for the receiving area or production floor of manufacturing facilities. In addition, Antaris™ FT-NIR analyzers (Thermo Nicolet Industrial Solutions, Madison, WI, USA) were designed to accommodate environments that would not be considered appropriate for normal laboratory equipment.

EXPERIMENTAL

Materials. α -Lactose monohydrate was obtained from Acros Chemical Company. α -Lactose anhydrous was purchased from Spectrum Chemical.

Preparation of Amorphous Lactose. To prepare the amorphous lactose, a 10% aqueous solution of α -lactose monohydrate was prepared and spray dried using a Yamoto Pulvis Mini Spray Drier (Yamoto Scientific Company, Tokyo, Japan). The following conditions were used:

Inlet air temp: 187°C
 Outlet air temp: 90°C (varied)
 Air flow rate: 0.34 m³/min

The material was further dried in a vacuum oven at 50°C for 30 minutes. The resulting amorphous material was dried over calcium sulfate.

Sample Preparation. Prior to mixture, the α -lactose monohydrate and α -lactose anhydrous were sieved to produce a consistent particle size range of 38 to 125 microns. The amorphous lactose was subjected to a sieving procedure to exclude particles above 150 microns. Use of larger particles for the latter cause agglomeration. XRPD showed that each of the starting materials contained only the appropriate forms in each case (see Figure 1). Binary mixtures of amorphous lactose and α -lactose monohydrate, amorphous lactose and anhydrous lactose, and α -lactose monohydrate and anhydrous lactose were prepared by weighing and mixing each

combination of materials on a mechanical shaker until they were uniform.

Ternary mixtures were prepared using α -lactose monohydrate, α -lactose anhydrous and amorphous lactose. These mixtures were prepared with the same screening procedures described above. Use of amorphous lactose contents greater than 40% (wt/wt) was avoided due to agglomeration. The experimental design for the preparation of the ternary mixtures is shown in Figure 2.

For each of the binary mixtures, two-thirds of the fifteen samples prepared were used for calibration while the rest were used as independent validation samples. For the ternary mixtures, three-fourths of the forty samples prepared were used for calibration while the rest were used as independent validation samples. This was possible because each sample was prepared independently. There were no "serial dilutions" employed during the preparation process. Samples used for validation were evenly dispersed throughout the range of the calibration.

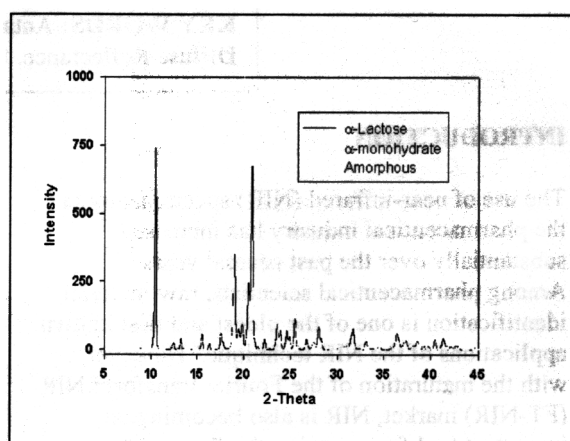


Figure 1. X-ray Powder Diffractograms for all three crystalline forms of lactose used as starting materials for this study

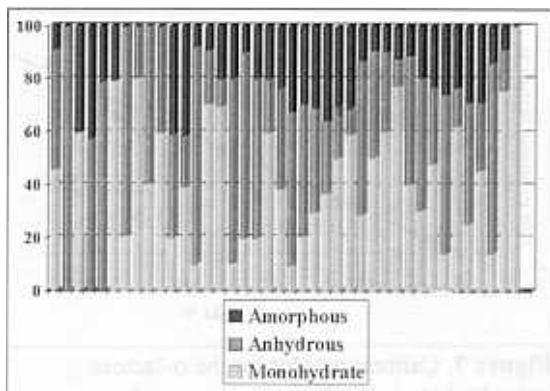


Figure 2. Phase diagram showing the experimental design for the ternary mixtures

FT-NIR Data Collection. FT-NIR spectra were collected for these samples on an Antaris FT-NIR analyzer. The integrating sphere module (Figure 3) that is standard to the instrument was employed. Room temperature conditions (21°C) were used. The data collection parameters for this experiment are listed below. Samples were placed in standard 2-dram vials for analysis. The data were collected through the bottom of each vial as in Figure 3.

Mode of Measurement: Reflectance
Spectral Range: 4000 cm^{-1} to 12,000 cm^{-1}
Resolution: 4 cm^{-1}
Co-Averaged Scans: 90
Data Collection Time: 67 seconds
Detector: InGaAs



Figure 3. The Antaris Solid Sampling system is configured in this photograph to collect reflectance data through the bottom of a sample vial filled with lactose powder

Chemometric Modeling. All chemometric modeling was performed using Thermo Nicolet's TQ Analyst™ software. The Partial Least Squares (PLS-I)* algorithm was used to derive calibration models. Predicted Residual Error Sum

of Squares (PRESS) plots were used to select the appropriate number of PLS factors for each model. Multiplicative Scatter Correction (MSC)⁵, Norris Derivatives⁶ and Savitzky-Golay⁷ derivatives were generally used for pre-treatment.

*TQ Analyst uses the PLS-I algorithm.

RESULTS AND DISCUSSION

Spectra for the three starting materials used in this study are shown in Figure 4. Second derivative plots of the three forms are shown in Figure 5. From these plots, it is clear that sufficient spectral differences exist to do quantitative characterization.

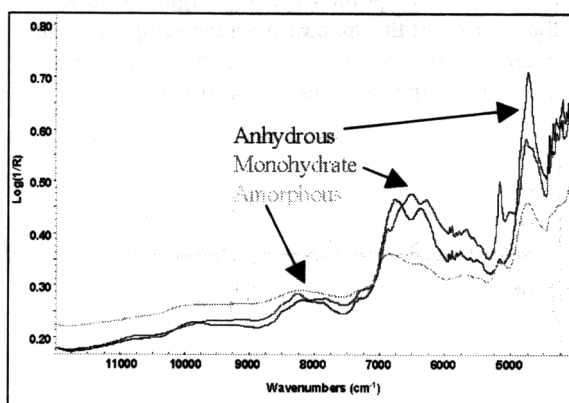


Figure 4. Full-range FT-NIR spectra for the three starting materials used in this study

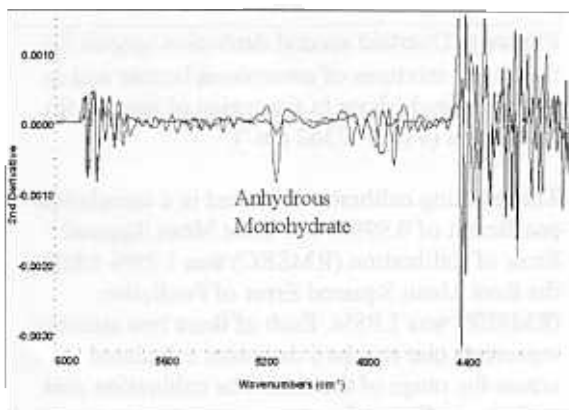


Figure 5. Full-range second derivative FT-NIR spectra for the three starting materials used in this study

Binary Mixtures. The data for the binary mixtures were examined first. This exercise was pursued to derive calibrations of each of these components under relatively simple conditions. This experimental design allowed determination of the best calibrations that could be constructed when lactose forms were mixed together.

The data for the amorphous lactose and α -lactose monohydrate mixtures were examined and a three-factor PLS equation was constructed. The calibration used data from the region 4482 to 7362 cm^{-1} , and the frequency range was selected with the TQ Analyst "Region Select" algorithm. MSC correction was applied followed by a Savitzky-Golay second derivative treatment (third-order, 17-point segment). Figure 6 shows the overlay of the spectra from the samples of this binary mixture following the specified treatments. The figure highlights the calibration region.

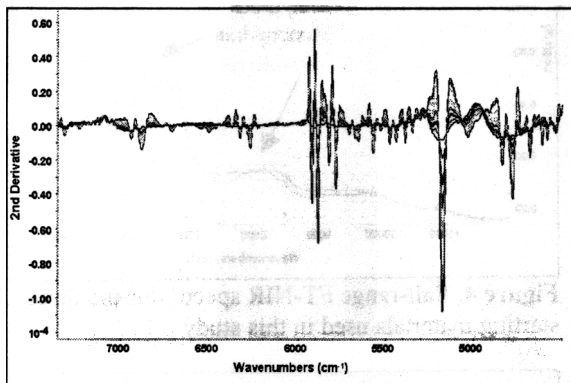


Figure 6. Overlaid second derivative spectra for the binary mixtures of amorphous lactose and α -lactose monohydrate in the region of interest for calibration (4482 – 7362 cm^{-1})

The resulting calibration resulted in a correlation coefficient of 0.9992. The Root Mean Squared Error of Calibration (RMSEC) was 1.29% while the Root Mean Squared Error of Prediction (RMSEP) was 1.98%. Each of these two statistics represents one standard deviation calculated across the range of the data. The calibration plot is shown in Figure 7.

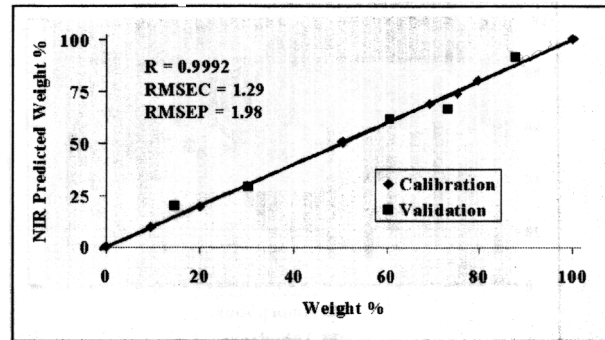


Figure 7. Calibration plot for the α -lactose monohydrate in the binary mixture with amorphous lactose

The data for the amorphous lactose and α -lactose anhydrous mixtures were examined and a three-factor PLS equation was constructed. The calibration was developed using the data from the range of 4287 to 4945 cm^{-1} . The frequency range was selected by the use of the TQ Analyst "Region Select" algorithm. A Norris second derivative (25-point segment, 0 gap) was employed. Figure 8 shows the overlay of the spectra in the region of interest for the samples of this binary mixture following the specified derivative process.

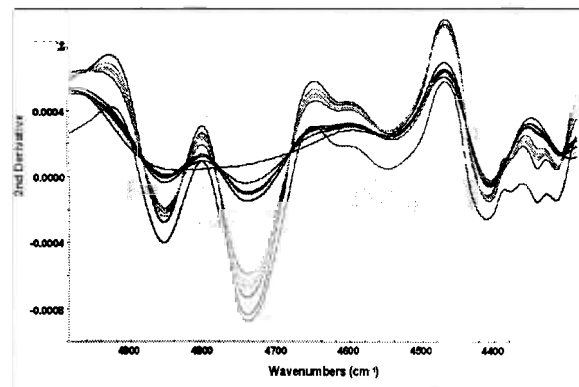


Figure 8. Overlaid second derivative spectra for the binary mixtures of amorphous lactose and α -lactose anhydrous in the region of interest for calibration (4287 – 4945 cm^{-1})

The calibration curve for the α -lactose anhydrous in the aforementioned binary mixture of amorphous lactose and α -lactose anhydrous is shown in Figure 9. A correlation coefficient of 0.9995 was obtained with an RMSEC of 1.05%. When the model was used to predict the validation samples, an RMSEP of 2.12% was achieved.

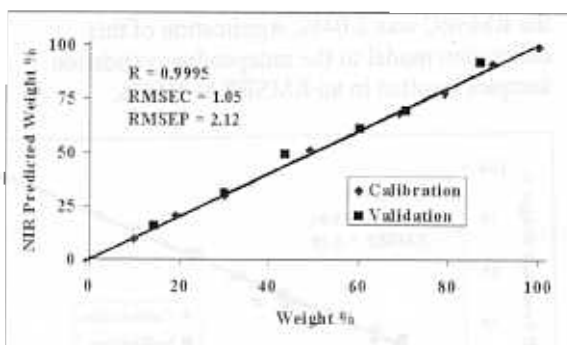


Figure 9. Calibration plot for α -lactose anhydrous in the binary mixture with amorphous lactose

The final binary mixture tested was composed of α -lactose anhydrous and α -lactose monohydrate. The spectral overlays for these samples are shown in Figure 10. In constructing a calibration model for this mixture, a three-factor PLS equation was used. The data were pre-treated using MSC followed by the use of a third-order Savitzky-Golay second derivative (13-point segment). With the aid of the TQ Analyst "Region Select" algorithm, the spectral region chosen for modeling was 4902 to 5768 cm^{-1} .

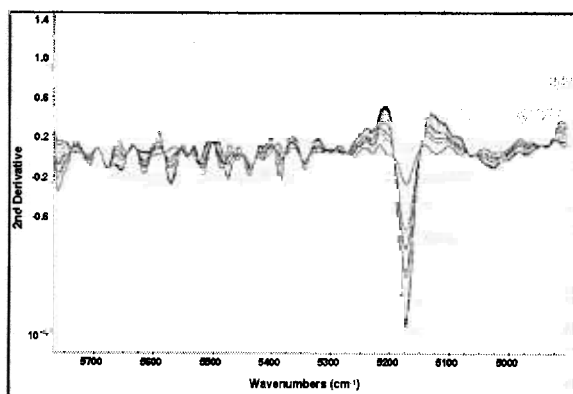


Figure 10. Overlaid second derivative spectra for the binary mixtures of α -lactose anhydrous and α -lactose monohydrate in the region of interest for calibration ($4902 - 5768$ cm^{-1})

The calibration curve for this third mixture shown in Figure 11 is based on the α -lactose anhydrous component. For this model, a correlation coefficient of 0.99999 was obtained with an RMSEC of 0.136% . When the model was applied to validation samples, an RMSEP of 0.718% was found. Quantification of the components in this mixture was much superior because the spectral response was better defined and much stronger for each of these components compared to the amorphous lactose, which was present in the

other two binary mixtures. In other words, the presence of amorphous lactose introduces a greater degree of difficulty in performing quantitative analyses in these types of mixtures.

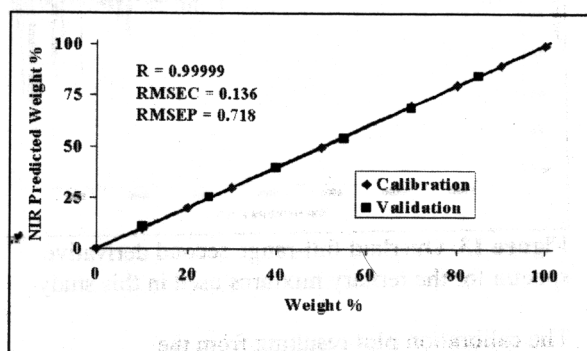


Figure 11. Calibration plot for α -lactose monohydrate in the binary mixture with α -lactose anhydrous

Ternary Mixtures. Because the results reported for the binary mixtures were encouraging, the next logical sequential step for this work was undertaken. Specifically, FT-NIR was applied to the quantitative characterization of ternary mixtures of the three forms of lactose. Extension of the work in this manner would come closer to meeting the true needs of those interested in this application since lactose received or produced as a specific form typically contains small but significant amounts of the other forms.

The overlay of the spectra for these ternary mixtures is shown in Figure 12. The same spectra are overlaid in Figure 13 after conversion to their respective second derivatives.

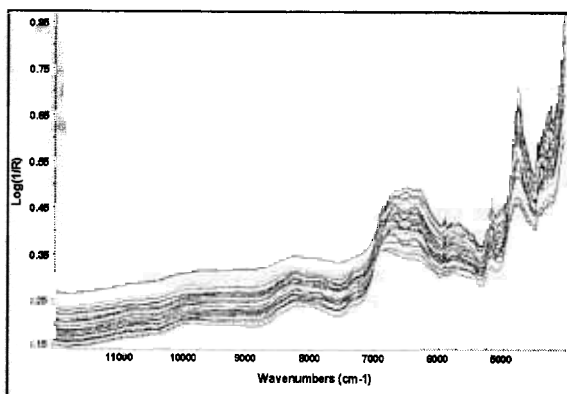


Figure 12. Full-range FT-NIR spectra for the ternary mixtures used in this study

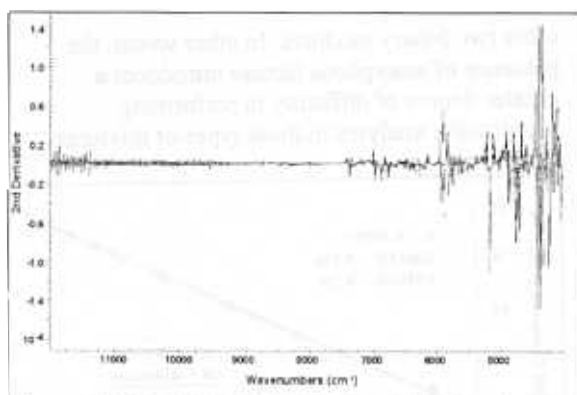


Figure 13. Overlaid full-range second derivative spectra for the ternary mixtures used in this study

The calibration plot resulting from the quantification model for α -lactose monohydrate is shown in Figure 14. A five-factor PLS model was employed using a frequency range from 5249 to 8916 cm^{-1} . The selection of the frequency range was aided by the use of the TQ Analyst "Region Select" feature. The data were pre-treated using a third-order Savitzky-Golay second derivative (25-point segment). The wide derivative filter was probably advantageous due to the use of the wide bands in the first and second overtone region for calibration.

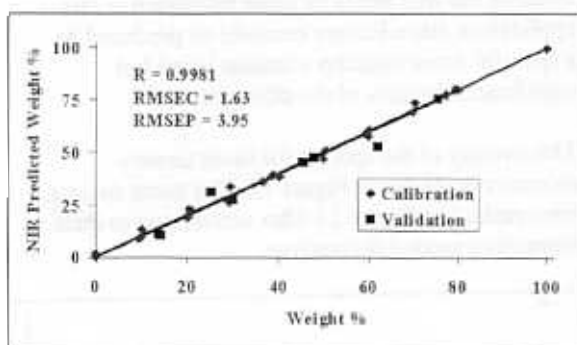


Figure 14. Calibration plot for the quantification of α -lactose monohydrate in the ternary mixtures

For the α -lactose monohydrate calibration, a correlation coefficient of 0.9981 was obtained with an RMSEC of 1.63%. When the calibration was applied to the independent validation samples, an RMSEP of 3.95% was achieved.

The calibration model for the α -lactose anhydrous in the ternary mixtures was also constructed using a five-factor PLS model with the same frequency range and data pre-treatment scheme employed for the α -lactose monohydrate calibration (see above). The calibration plot is shown in Figure 15. The correlation coefficient was 0.9971 while

the RMSEC was 2.04%. Application of this calibration model to the independent validation samples resulted in an RMSEP of 3.40%.

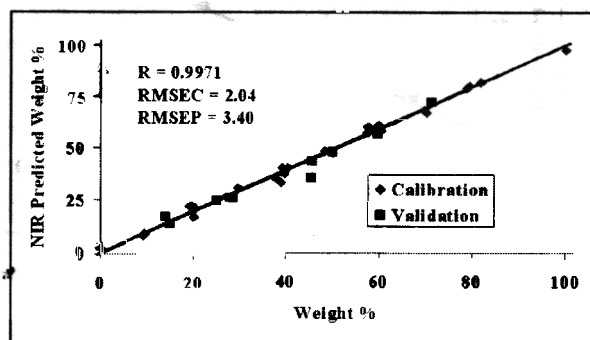


Figure 15. Calibration plot for the quantification of α -lactose anhydrous in the ternary mixtures

The calibration model for the amorphous lactose content in the ternary mixtures used a three-factor PLS model with the same frequency range and data pre-treatment scheme employed for the previous two components (see above). The calibration plot is shown in Figure 16. Fewer factors were used compared to the other two calibrations due to the instability associated with the use of more factors (revealed by the PRESS plot). It is likely that the amorphous lactose calibration was constructed to a large extent using the spectral information related to the other two components. In other words, as the amorphous content increased, the *absence* of spectral intensity related to the other two components probably contributed significant information to the calibration model development.

The correlation coefficient for the amorphous lactose model was 0.9817 while the RMSEC was 2.26%. An RMSEP of 2.02% was achieved when the model was applied to the validation samples. Compared to the other two components, the correlation coefficient was affected to some extent by the smaller calibration range (see Experimental Section). The RMSEP was smaller than the RMSEC because the validation samples spanned about one-half of the range of the calibration samples. As seen in Figure 16, the validation samples were spread through the middle of the range.

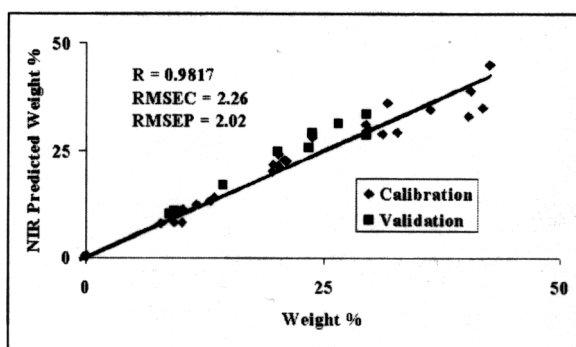


Figure 16. Calibration plot for the quantification of amorphous lactose in the ternary mixtures

It was expected, based on the work with the binary mixtures, that the results for the quantification of the two crystalline forms, α -lactose anhydrous and α -lactose monohydrate, would be superior to the results for the amorphous content. It was generally true that better calibrations were obtained for the two crystalline forms compared to the amorphous form. Because the NIR response for the crystalline materials was superior by comparison, slightly better errors could be achieved over larger ranges with these calibration models.

The limits of quantification (LOQs) were calculated for each of the components by applying the calibration models to replicate measurements of samples with low (less than 1%) contents of the respective components (see Table 1). A standard deviation was calculated and multiplied by three to estimate the limits of quantification. The following results were obtained:

Table 1. Estimated Limits of Quantification by the Standard Method Deviation

α -Lactose Monohydrate	– 4.1%
α -Lactose Anhydrous	– 5.2%
Amorphous Lactose	– 6.4%

Generally, most NIR practitioners use real lots of incoming materials or real production materials to produce calibrations. In this case, we have used synthetic materials to produce the calibrations reported. A legitimate question can be posed about the applicability of these models to real-life samples. Although this issue has not been directly addressed with these materials, a similar experiment performed previously by this group⁸ suggests there is legitimacy in this approach. Figure 17 shows the results of an experiment in which synthetic samples were prepared containing varying amounts of crystalline and

amorphous sucrose. A calibration was prepared from these mixtures and was applied to real, homogeneous samples with varying amounts of each type of sucrose. The homogeneous samples were prepared by milling crystalline sucrose for prescribed amounts of time. The figure shows the results of the application of two different NIR calibrations compared to the results of the XRPD analyses for the same samples. Although there is a bias compared to the XRPD results, the NIR calibration appears to give similar answers. A bias can easily be explained and adjustments can be made accordingly. The critical point of interest is that the pattern for the XRPD results is generally followed by the NIR results. This suggests that a protocol such as the one followed here to show feasibility represents a legitimate approach to this problem.

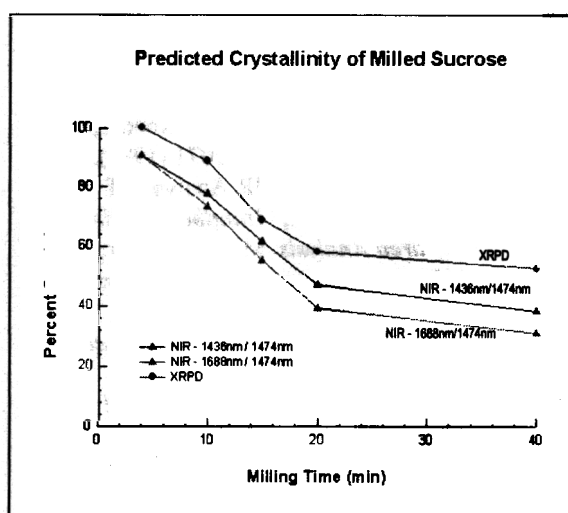


Figure 17. Comparison of predictions of milled sucrose amorphous content by X-ray Powder Diffraction and two different NIR calibrations. This figure shows the legitimacy of using NIR calibrations constructed from binary mixtures and then applying such calibrations to homogeneous systems

CONCLUSION

The data shown in this report suggest that FT-NIR can be used to simultaneously characterize the content of amorphous lactose, α -lactose anhydrous and α -lactose monohydrate present in the same sample. It was first demonstrated that calibrations could be constructed for binary mixtures of various combinations prepared from the three forms of lactose. Then, quantification of the various forms of lactose was successfully achieved in a co-varying ternary matrix. The

limits of quantification in the ternary mixtures ranged from 4.1% to 6.4%. Data were also presented suggesting the synthetic systems used in this work are applicable to real samples.

Based on the data presented in this report, it can be concluded that FT-NIR is a useful tool for the characterization of the crystalline status of raw materials or production samples. This is a very useful capability since the presence of crystalline contaminants can affect the success of the production process and the performance and/or stability of the final product.

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