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# Quantifying Low Amorphous or Crystalline Amounts of Alpha-Lactose-Monohydrate Using X-Ray Powder Diffraction, Near-Infrared Spectroscopy, and Differential Scanning Calorimetry

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## ABSTRACT

Efficient and accurate quantification of low amorphous and crystalline contents within pharmaceutical materials still remains a challenging task in the pharmaceutical industry. Since X-ray powder diffraction (XRPD) equipment has improved in recent years, our aim was 1) to investigate the possibility of substantially lowering the detection limits of amorphous or crystalline material to about 1% or 0.5% w/w respectively by applying conventional Bragg Brentano optics, combined with a fast and simple evaluation technique; 2) to perform these measurements within a short time to make it suitable for routine analysis; and 3) to subject the same data sets to a partial least squares regression (PLSR) in order to investigate whether it is possible to improve accuracy and precision compared to the standard integration method. Near-infrared spectroscopy (NIRS) and differential scanning calorimetry (DSC) were chosen as reference method. As model substance, alpha lactose monohydrate was chosen to create calibration curves based on predetermined mixtures of highly crystalline and amorphous substance. In contrast to DSC, XRPD and NIRS revealed an excellent linearity, precision, and accuracy with the percent of crystalline amount and a detectability down to about 0.5% w/w. Chemometric evaluation (partial least squares regression) applied to the XRPD data further improved the quality of our calibration.

*Key Words:* X-ray powder diffraction; Near-infrared spectroscopy; Differential scanning calorimetry; Amorphous; Crystalline; Lactose; Quantification; PLS regression.

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## INTRODUCTION

It is increasingly important to characterize the degree of crystallinity in pharmaceutical materials in order to allow better quality products to be produced.[1] Subtle differences in the crystal order can already give rise to variations among batches or sources of the same chemical substance, which may affect the performance of products, either during further processing (flow, mixing, compression) or storage of the final formulation.[2–6]

As a consequence, the formation or recrystallization of amorphous phases should be monitored regularly. This requires an analytical technique that is accurate, fast, and nondestructive and therefore suitable for routine analysis in pharmaceutical industry. Gravimetric and calorimetric methods like dynamic vapor sorption,[7,8] solution calorimetry,[9,10] or isothermal calorimetry[11] have proved capable of detecting amorphous material down to about 1% or even lower but are in general either time consuming or destructive. The same is valid for some spectroscopic techniques such as Fourier-transform (FT) infrared spectroscopy[12] or solid-state nuclear magnetic resonance (SS-NMR).[13] Only FT near infrared,[8,14–16] FT Raman,[17] and X-ray powder diffraction (XRPD)[7,12,18–21] have successfully been able to quantify crystallinity in different materials within an acceptable time, allowing complete sample retrieval and accurate results. For near-infrared spectroscopy (NIRS), the detection limit was estimated to be around 1% for amorphous as well as crystalline material utilizing the ratio of two wavelengths,[14] inverted least squares regression,[15] or multiple linear regression (MLR)[8,16] as evaluation method. The time of measurement was in the range of minutes. Raman spectroscopy also detected crystalline and amorphous material at levels of approximately 1% within a similar time scale.[17] For XRPD, different results regarding the detection limit or precision were observed depending on the applied data evaluation technique. The residual amount of amorphous sucrose in its crystalline matrix was examined by calculating the ratio of the integrated peak intensity of crystalline sucrose at 18.8°2θ to the peak intensity of the internal standard LiF at 45°2θ. In these measurements Saleki-Gerhardt, Ahlneck, and Zografi[7] reached a minimum quantifiable level of about 10% (w/w). In contrast, the detectability of residual crystalline sucrose in its amorphous matrix was determined to be 0.9% (w/w).[19] Gombás et al.[16] evaluated XRPD data by MLR on four specific diffraction peaks and were able to detect 5% amorphous lactose in a physical mixture with crystalline alpha-lactose monohydrate and vice versa. Crocker and McCouley[20] investigated the crystallinity of Imipenem samples by measuring the X-ray intensity diffracted by amorphous material at one specific diffraction angle where no crystalline peak was present in order to avoid texture problems. The estimated precision of this approach was +/−5%. The groups mentioned above utilized the conventional Bragg-Brentano focusing optics. In contrast, Chen, Bates, and Morris[18] applied parallel beam XRPD in order to eliminate errors due to differing sample height or surface roughness, but accepted low resolution and intensity instead. The diffractograms were normalized to the same total intensity and then evaluated by whole pattern fitting. The limit of detection was calculated to be 0.37% (w/w). The time of measurement was in general at least 10–20 min per sample and therefore too long for routine analysis. Since XRPD equipments have improved in recent years, our aim was 1) to investigate the possibility of substantially lowering the detection limits of amorphous or crystalline material down to about 1% or 0.5% w/w without applying special optics[18] or complex evaluation methods,[18,21] but using conventional Bragg Brentano optics and a simple evaluation technique; 2) to perform these measurements within a short time to make it suitable for routine analysis; and 3) to subject the same data sets to a partial least squares regression (PLSR) in order to investigate whether it is possible to improve accuracy and precision compared to the standard integration method. Near-infrared spectroscopy and DSC were chosen as reference methods.

## EXPERIMENTAL

### Materials and Sample Preparation

Alpha-lactose-monohydrate (Granulac 200, Meggle AG, Wasserburg, Germany) was chosen as the model substance since it is a commonly used excipient in the pharmaceutical industry. Its crystalline forms or the presence of an amorphous fraction can strongly influence the resulting tableting properties and hence the performance of the finished product (tensile strength or dissolution rate).[22,23]

The delivered lactose-monohydrate was already milled and had an average particle diameter of about $x_{50}$: 25 μm ($x_{10}$: 3 μm, $x_{90}$: 70 μm), determined by laser diffractometry (Helos LF, Sympathec, Clausthal-Zellerfeld, Germany, Rhodos dispersing system). Samples to be taken 100% crystalline were stored at a relative humidity of 65% and 30°C for 1 week to ensure a complete recrystallization of any amorphous

regions present [50–60% relative humidity (RH) is the critical RH for crystallization of amorphous lactose].[24]

Samples of amorphous lactose ($x_{10}$: 3.5 μm, $x_{50}$: 14 μm, $x_{90}$: 64 μm) were prepared by lyophilization of an aqueous saturated lactose solution. The solution was frozen in liquid nitrogen (−196°C) and then left for 48 hours in a freeze dryer (Christ Alpha 2–4, Christ Gefriertrocknungsanlagen, Osterode, Germany). After that, the temperature was gradually increased to 50°C to ensure that the resulting product was entirely dry. The lyophile was removed from the freeze dryer and stored in a desiccator over silicagel (0.2% RH). The amorphous state was confirmed by XRPD (Fig. 1) and the residual water content (<0.5% w/w) was determined by TGA. Spectra (XRPD, NIRS, and DSC) were obtained of the pure crystalline and amorphous form and of binary mixtures in intervals of 10% (10–90% amorphous content), 1% (0–10% amorphous content), or 0.5–2% (90%, 93%, 95%, 97%, 99%, 99.5%, 100% amorphous content). At least three replicate measurements were performed for each sample in each technique. For all methods the same sample sets were used and run nearly simultaneously. The preparation of the mixtures was done as follows: each constituent was accurately weighed and then thoroughly mixed with the others in an agate mortar with a pestle. After that, the mixtures were stored at a relative humidity of about 0.2% at room temperature to prevent recrystallization of the amorphous part. All mixtures were prepared out of the same batches of crystalline and amorphous lactose, respectively.

## Methods

### X-Ray Powder Diffraction

According to the backloading technique, about 400 mg of sample was pressed evenly by hand into a circular sample holder (PW 1811/00+PW 1811/16, PANalytical B.V., Almelo, The Netherlands) using a special powder press block (PW 1770/10, PANalytical B.V., Almelo). By doing this, the sample surface gets flat, smooth, and flush with the sample holder surface. Furthermore, preferred orientation is minimized and good reproducibility is achieved. In order to improve particle statistics, the sample was spun around its y-axis with one revolution per second during an XRD run. The sample preparation and measurements were performed at ambient temperature and 20–30% RH. X-ray diffraction patterns were obtained using an X'Pert PRO MPD system (PANalytical B.V., Almelo, the Netherlands), which worked with Ni-filtered Cu-Kα radiation (40 kV, 40 mA), programmable divergence slit, fixed mode (1/4°), Bragg-Brentano focusing optics, and the X'Celerator as detector.

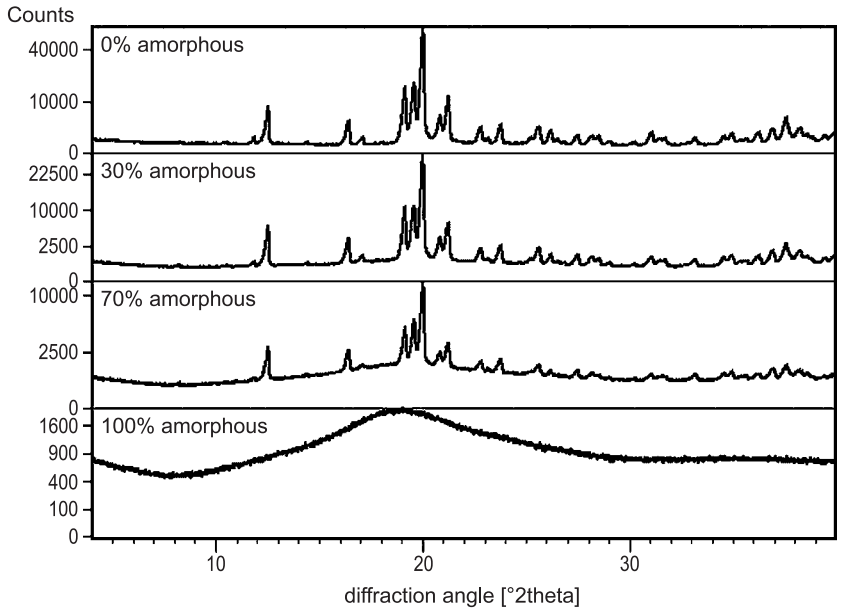


***Figure 1.*** X-ray diffractograms of samples containing different amorphous contents (0%, 30%, 70%, and 100%).

The following measuring conditions were applied:

Mixture 0—100%: 0.008 step size, 3 min per measurement, 4–40°2θ
Mixture 1—9%: 0.016 step size, 2 min per measurement, 7–45°2θ
Mixture 90—100%: 0.008 step size, 6 min per measurement, 12–13°2θ

The evaluation of the data was done with PANalytical's X'Pert HighScore® software for phase identification (classical analysis) as well as with the Unscrambler® software (multivariate analysis, PLS regression using cross validation, 3er segments).

### Near-Infrared Spectroscopy

About 2 g of sample was placed in a glass vial and reflectance spectra were taken with a Vector 22/N FT NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) by immersing the fiber optics into the powder. The NIR instrument recorded the mean spectrum of 32 scans of each sample over a wave number region of 3800–12000 $cm^{-1}$, which took 30 sec. The regression model for the samples was calculated by PLS after data preprocessing by multiplicative scatter analysis and first derivative (Savitzky-Golay differentiation, 2nd polynomial order, +/−3 averaging) using the Unscrambler software. The wave number region of 5342–4865 $cm^{-1}$ (OH stretching combination band) was taken for the evaluation of mixtures with 10–90% crystalline and 0–10% amorphous content, whereas mixtures with 0–10% crystalline content were evaluated in the wave number region of 5992–5693 $cm^{-1}$, which is independent from the water content of the sample.

### Differential Scanning Calorimetry

Measurements of the crystallization energy were carried out on a Perkin Elmer Pyris 1 (Boston, USA) differential scanning calorimeter using nonhermetically crimped aluminum pans at a scanning rate of 10°C per minute. About 3.5 mg of each lactose sample was weighed in each pan. The evaluation was done by estimating the area under the exothermic crystallization peak.

### Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was carried out on a Perkin Elmer Thermogravimetric Analyser TGA 7, which was connected to a Perkin Elmer Thermal Analysis Controller TAC 7/DX. About 3–4 mg of the pure amorphous and the pure crystalline lactose was weighed in an open platinum pan and heated from room temperature to 185°C at 10°C/min under a stream of nitrogen.

### Error Estimation

In order to get an estimate of the errors connected with XRPD and NIRS a couple of measurements were made:

- The instrument reproducibility was determined by measuring a sample of pure crystalline α-lactose-monohydrate 10 times without removing it from the instrument.
- Variations due to different sample packing were estimated by repacking the same sample of α-lactose-monohydrate into the sample holder 10 different times and collecting the corresponding data sets.
- The day-to-day reproducibility was observed for 4 days using a single mixture of a sample (4% w/w).
- The method error was assessed by analyzing the 4% w/w sample six times in succession. Between the measurements the sample was repacked.

Based on the calibration curves, the crystallinity for each measurement was predicted and the standard deviation (%) was calculated.

### Model Validation

In order to validate the calibration results of the PLSR, an internal validation procedure was applied the so called cross validation. The same samples are used for both model estimation and testing. A few samples containing the same amount of amorphous material are left out from the calibration data set and the model is recalibrated. Then the values for the left-out samples are predicted and the prediction residuals are computed. This process is repeated with all other subsets, too, until every set has been left out once. After that, the prediction residuals are combined to get the validation residual variance and the root mean square error of prediction (RMSEP).[25]

### Estimation of Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ were determined from multiple measurements of samples containing a concentration of

crystalline or amorphous lactose nearby the estimated LOD, i.e., 0.5% w/w or 1% w/w, respectively. For the classical evaluation of the XRPD data using $I_{net}/I_{obs}$ ratios or the peak area (see results), the LOD and LOQ were calculated as follows:[12]

$$\text{LOD} = 3 \times \text{sd} / \text{slope of the corresponding calibration curve}$$

$$\text{LOQ} = 10 \times \text{sd} / \text{slope of the corresponding calibration curve}$$

For the standard curves based on PLSR:[14]

$$\text{LOD} = 3 \times \text{sd}$$

$$\text{LOQ} = 10 \times \text{sd}$$

## RESULTS AND DISCUSSION

### X-Ray Powder Diffraction

Figure 1 shows the X-ray diffraction patterns of the pure amorphous and the pure crystalline form and of physical mixtures with 30% and 70% amorphous content. With an increasing amount of amorphous material, the base line increases, too, whereas the peaks, which are a measure for the crystalline content, decrease. Therefore, the evaluation of the crystalline content is done using the profile fitted area under the peak at 12.4°2θ, whereas the amorphous content is evaluated by calculating the ratio of the net intensity of all peaks to the observed intensity over the whole range [$I_{net}/I_{obs;}$ ''Hermans' method:''[19,21] $I_{net}$ corresponds to the intensity, diffracted by the crystalline phase and $I_{obs}$ to the total intensity which is a sum of the intensities of the crystalline and the amorphous phase and the natural, in general, constant background ''noise'']. The background was approximated by an iterative method according to Sonneveld and Visser with a bending factor of zero. The peak at 12.4°2θ was chosen as analytical peak because it showed fewer variations than the other peaks. Furthermore, most of the recrystallization products of amorphous lactose [mainly α-lactose-monohydrate, but also stable and unstable anhydrous α-lactose and anhydrous crystals with α and β lactose in varying molar ratios][26] have a diffraction peak at this position.

The resulting calibration curves for the different mixtures (10–90%, 1–9%, and 90–100% amorphous content) are given in Fig. 2A–C. Each data point is the average of at least three independent measurements. All curves show an excellent linearity between the peak area or $I_{net}/I_{obs}$ ratio respectively, and the crystalline or amorphous content. The correlation coefficients, the limits of detection and quantification and the values for precision and bias are summarized in Table 1a–c. Precision and bias are a measure for the

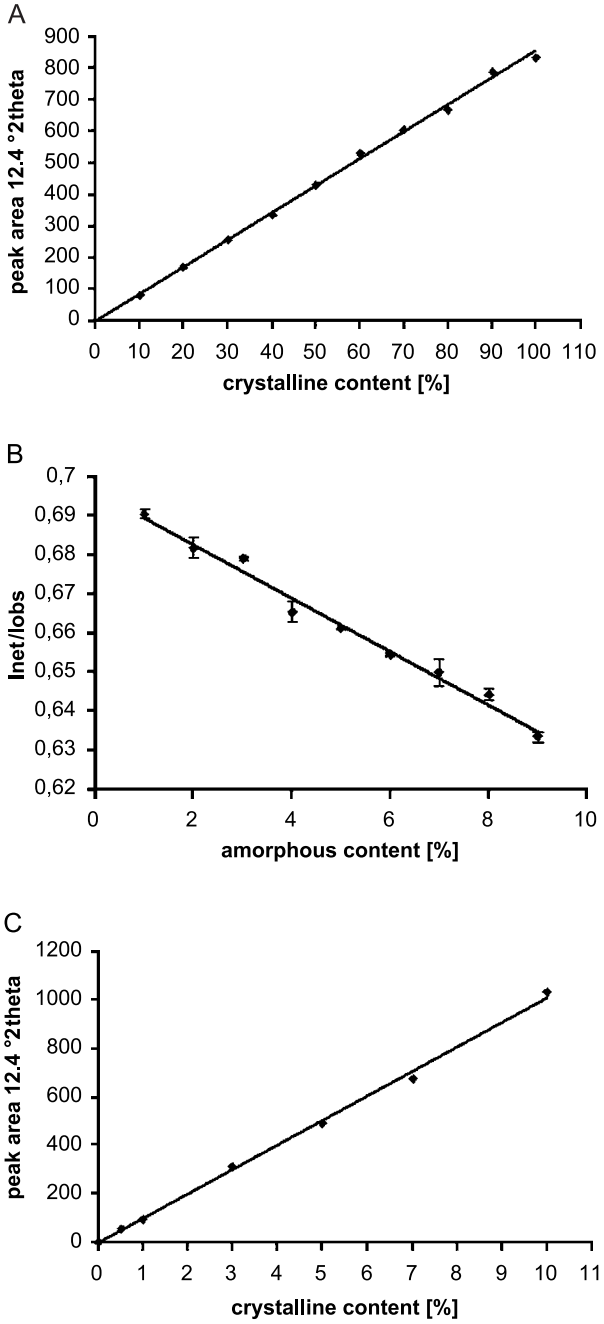


*Figure 2.* (A) XRPD calibration curve 10–90% crystalline content. (B) 1–9% amorphous content. (C) 0–10% crystalline content.

***Table 1.*** (a) Statistical results of the calibration curve 10–90% amorphous content; (b) 1–9% amorphous content; (c) 0–10% crystalline content.

| Ideal content (%) | Actual content (%) | sd (%) (n=3) | Bias (%) |
|---|---|---|---|
| *a* | | | |
| 10 | 9.56 | 0.62 | −0.44 |
| 20 | 19.56 | 0.45 | −0.44 |
| 30 | 30.17 | 0.77 | 0.17 |
| 40 | 39.34 | 0.85 | −0.66 |
| 50 | 50.21 | 1.32 | 0.22 |
| 60 | 62.22 | 2.73 | 2.23 |
| 70 | 70.81 | 2.35 | 0.81 |
| 80 | 78.31 | 2.11 | −1.69 |
| 90 | 92.36 | 1.65 | −2.36 |
| Corr. coeff. | 0.999 | | |
| *b* | | | |
| 1 | 0.86 | 0.31 | −0.14 |
| 2 | 2.15 | 0.76 | 0.15 |
| 3 | 2.53 | 0.15 | −0.47 |
| 4 | 4.51 | 0.76 | 0.51 |
| 5 | 5.14 | 0.06 | 0.14 |
| 6 | 6.12 | 0.13 | 0.12 |
| 7 | 6.81 | 1.01 | −0.19 |
| 8 | 7.63 | 0.42 | −0.37 |
| 9 | 9.2 | 0.19 | 0.2 |
| Corr. coeff. | 0.994 | | |
| LOD (%) | 0.93 | | |
| LOQ (%) | 3.10 | | |
| *c* | | | |
| 10 | 10.23 | 0.38 | 0.23 |
| 7 | 6.71 | 0.39 | −0.29 |
| 5 | 4.89 | 0.21 | −0.11 |
| 3 | 3.09 | 0.16 | 0.09 |
| 1 | 0.99 | 0.08 | −0.01 |
| 0.5 | 0.56 | 0.15 | 0.06 |
| Corr. coeff. | 0.999 | | |
| LOD (%) | 0.45 | | |
| LOQ (%) | 1.51 | | |

systematical and random error and were determined within a day using following formula:

$$\text{Bias} = \bar{x} - x_{true}$$

standard deviation :

$$\text{sd}\ (\%) = \sqrt{\frac{\sum_{1}^{n}(x_1[\%] - \bar{x}[\%])^2}{n-1}}$$

It is obvious that it is perfectly suitable to quantify a residual amorphous or crystalline content accurately by XRPD using conventional Bragg-Brentano optics together with simple and fast evaluation methods. However, comparing the precision, it is apparent that the XRPD measurements show lower standard deviations when estimating a crystalline content compared to the quantification of low amorphous contents. The time of measurement is about 2 minutes for low amorphous contents (1% w/w) and 6 minutes for low crystalline (0.5% w/w) contents. For higher residual amounts this time can be further reduced to 1 or 3 minutes per measurement, respectively.

Alternatively to this ''classical'' evaluation method of the XRPD data, chemometric evaluation was performed using PLS regression in order to investigate the possibility of improving accuracy and precision compared to the standard evaluation technique. During

*Table 2.* Statistical results of the chemometric evaluation of the XRPD data.

| | 10–90% Crystalline | 0–10% Crystalline | 1–9% Amorphous | |
|---|---|---|---|---|
| Used X-variables | Background level | 12–13°2theta | Background level | 10–35°2theta |
| Correlation Cal | 0.9996 | 0.9996 | 0.996 | 0.998 |
| Correlation Val | 0.9995 | 0.9993 | 0.991 | 0.992 |
| RMSEC | 0.916 | 0.099 | 0.231 | 0.151 |
| RMSEP | 0.998 | 0.136 | 0.333 | 0.379 |
| SEC | 0.931 | 0.102 | 0.236 | 0.154 |
| SEP | 1,014 | 0.136 | 0.338 | 0.384 |
| Bias Cal | 4.17E-07 | 3.63E-07 | 8.83E-08 | −1.68E-07 |
| Bias Val | −0.025 | −0.031 | 0.024 | 0.035 |
| Number of PCs | 1 | 2 | 2 | 3 |
| LOD (%) | nd | 0.27 | 0.27 | 0.57 |
| LOQ (%) | nd | 0.90 | 0.90 | 1.90 |

Note: nd: not determined.

PLS regression the X and the Y matrices are both modeled simultaneously in order to find the latent variables in X that will predict the Y variable the best. Two different approaches were chosen, either using the base line as X variables or the peaks and the base line. The results of the regressions are presented in Table 2. The first approach, which only models variations within the base line, is based on the assumption that particle size effects and therefore preferred orientation or microabsorption effects do not have an influence on this part of the spectrum but have major effects on the peaks. This hopefully guarantees that later lactose samples can be analyzed independently of particle size and shape. The second approach, which uses the peaks and the base line, contains additional information, as a growing amorphous content not only influences the base line but also the shape of the peaks. On the other hand, this calibration is very sensitive to texture effects. In contrast to NIRS, where particle size effects can be minimized by data preprocessing like MSC, this cannot be used for XRPD, as it would eliminate informative variations within the spectra that correlate with the amorphous content. For the determination of low crystalline contents, the variation of the (110) reflection was modeled.

The results of the chemometric evaluation also show an excellent linearity with similar correlation coefficients as the classical evaluation. Compared to the latter, the calibration as well as the cross validation model is more stable and accurate, which can be seen at the root mean square error (RMSE), the standard error (SE), the bias, and the LOD or LOQ, respectively (Table 2). The RMSE is calculated for the predicted or for the calibration samples and is a measure for the average difference between predicted and measured response values at the validation (RMSEP) or the calibration stage (RMSEC).[12] The lower the values, the higher the predictive accuracy. For the validation model with 10–90% intervals the certainty of prediction is +/−1%, for the one modeling the low crystalline contents it is about +/−0.15%, and for the one with 1–9% amorphous content it is 0.33% (background level was modeled) or 0.38% (peaks and background were modeled). The values for the LOD and the LOQ indicate that the quantification of the amorphous content using just the baseline is advantageous (LOD: 0.27%) compared to the combination of peaks and baseline (LOD: 0.5%).This can also be seen at the number of PCs needed to obtain a good model: for the peak-baseline combination one more PC is necessary to model variations due to differing peak

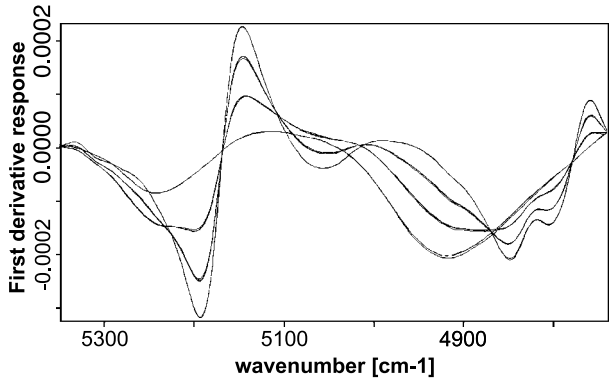


*Figure 3.* NIR Spectra (OH combination band) of samples containing 0%, 30%, 70%, and 100% amorphous lactose after multiplicative scatter correction and first derivative.

*Table 3.* Statistical results of the chemometric evaluation of the NIRS data.

| | 10–90% Crystalline | 0–10% Crystalline | 1–9% Amorphous |
|---|---|---|---|
| Used X-variables | 5342–4865 cm−1 | 5992–5693 cm−1 | 5342–4865 cm−1 |
| Correlation Cal | 0.9993 | 0.9963 | 0.9945 |
| Correlation Val | 0.9985 | 0.9904 | 0.9926 |
| RMSEC | 1,176 | 0.296 | 0.33 |
| RMSEP | 1,785 | 0.537 | 0.396 |
| SEC | 1,194 | 0.303 | 0.335 |
| SEP | 1,812 | 0.542 | 0.402 |
| Bias Cal | 2.31E-06 | 1.36E-07 | 3.83E-07 |
| Bias Val | 5.80E-02 | −9.40E-02 | −1.70E-02 |
| Number of PCs | 3 | 2 | 1 |
| LOD (%) | nd | 0.75 | 0.2 |
| LOQ (%) | nd | 2.5 | 0.65 |

Note: nd: not determined.

heights. The results indicate that this novel approach to the evaluation of the XRPD data implies a further improvement in accuracy and detection limit of amorphous and crystalline contents compared to the previous results.[18,19]

$$\text{RMSEP} = \sqrt{\frac{1}{N} \cdot \sum_{n=1}^{N} \left(y_n^{pred} - y_n^{true}\right)^2}$$

$$\text{SEP} = \sqrt{\frac{1}{N-1} \cdot \sum_{n=1}^{N} \left(y_n^{pred} - y_n^{true} - Bias\right)^2}$$

$$\text{Bias} = \frac{1}{N} \sum_{n=1}^{N} \left(y_n^{pred} - y_n^{true}\right)$$

$$\text{N} = \text{number of samples}$$

## Near-Infrared Spectroscopy

According to XRPD, three replicates of each sample were taken. Figure 3 shows the first derivative of the OH stretching combination band after multiplicative scatter correction (MSC) for samples with 10%, 30%, 70%, and 100% amorphous content. The MSC was used in order to compensate for multiplicative and additive scatter effects in the data (e.g., caused by particle size effects), and the first derivative was taken to sharpen spectral features for better extraction of information. In contrast to other groups, who used MLR as regression method for the NIR data,[8,16] a PLS regression was chosen for the evaluation of our data. As NIR data are known to be highly redundant and hence show intercorrelations between the X-variables, it is not recommended to use calibration methods that assume each X-variable to have unique information about Y like MLR. By PLS, the information of the many observed X-variables is linked to a few underlying variables, which leads to an easier interpretable model, leaving out much of the noise and solving colinearity problems. Furthermore the PLS method uses all of the data to extract quantitative information, whereas MLR utilizes just a few spectral data points.[27] Table 3 shows the regression results.

Similar to the XRPD results, NIRS also reveals an excellent linearity with correlation coefficients better than 0.99. The RMSEC and RMSEP values indicate that the predictive accuracy of NIRS is slightly lower compared to XRPD but still very good. The LOD shows that NIRS is slightly superior to XRPD in detecting low amorphous contents [0.2% w/w (NIRS) vs. 0.27% w/w (XRPD)], whereas XRPD is more capable of detecting low crystalline contents [0.27% w/w (XRPD) vs. 0.4% w/w (NIRS)].

## Differential Scanning Calorimetry

Figure 4 shows the calibration curve obtained by estimating the area under the crystallization peaks. The crystallization energy ΔH is plotted vs. the weight fraction of amorphous lactose. As the crystallization exotherms of the mixtures all appear approximately at the same temperature (104°C), no correction of the measured ΔH is necessary. The α-lactose-monohydrate doesn't show any temperature-induced transitions at this temperature and therefore doesn't falsify the measured enthalphy there. Only the crystallization exotherm for the pure amorphous substance appeared at a higher temperature (about 145°C), due to lacking

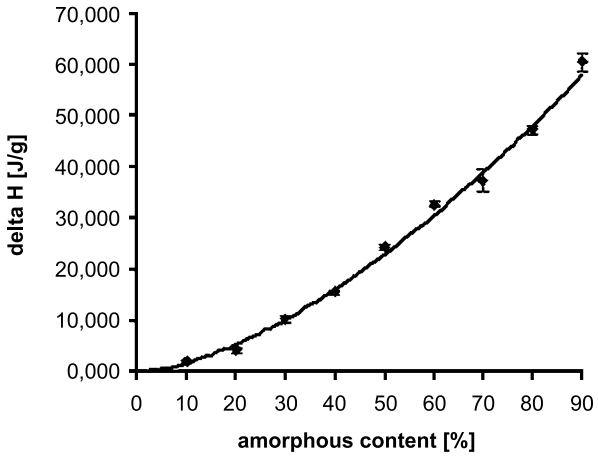


*Figure 4.* DSC calibration curve, 10–90% amorphous content.

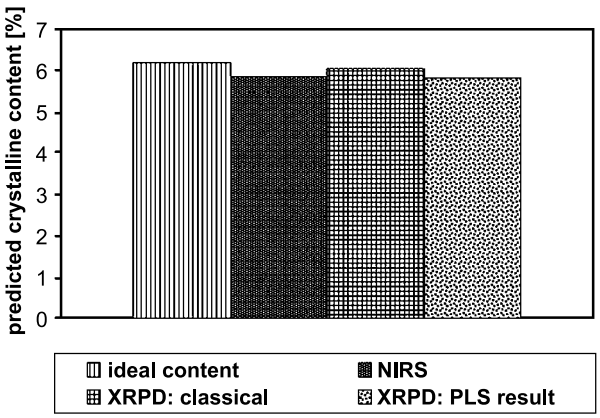


*Figure 6.* Verification of the calibration curve (XRPD) and validation models (XRPD, NIRS), 0–10% crystalline content.

crystallization nuclei that were already present within the mixtures. Consequently, this value wasn't included in the calibration. Unfortunately, the DSC results do not have a linear behavior. Though the fitted polynome is quite good (correlation coefficient of 0.9914), it indicates a low sensitivity (i.e., a low slope) for small amorphous contents. The lack of linearity may be due to an incomplete crystallization of the amorphous part or due to the fact that only sealed and not hermetically sealed pans were used. Furthermore, the onset and rate of crystallization are strongly dependent on the contact surface of the material to the bottom of the pan with the consequence that the heat transfer varies. Because of this nonlinearity, the DSC was not utilized for further investigations, LOD and LOQ weren't calculated, and no assessment of the possible errors was made. Nevertheless, other groups that used modulated DSC or conventional DSC with hermetically sealed samples obtained better results.[7,28]

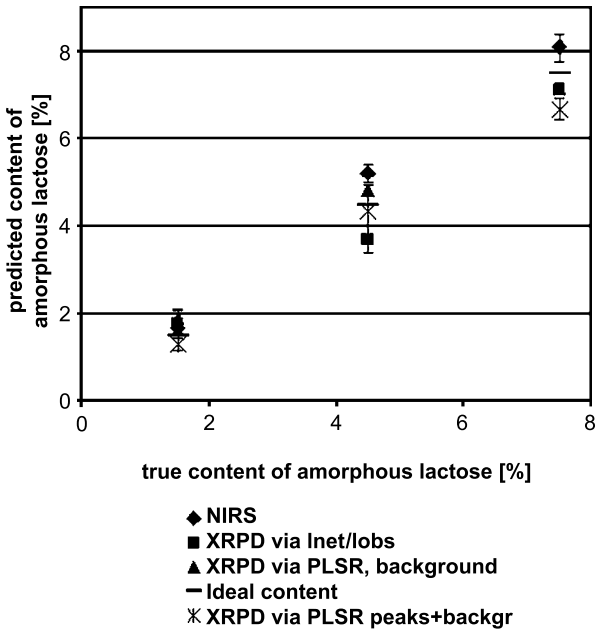


*Figure 5.* Verification of the calibration curve (XRPD) and validation models (XRPD, NIRS), 1–9% amorphous content.

## Test Samples

In addition to the cross validation procedure, four new physical mixtures were prepared, three of them contained small amounts of amorphous lactose (1.5%, 4.5%, and 7.5% w/w), and one contained a low crystalline content (6.2% w/w) in order to verify the PLS models. The results are shown in Figs. 5 and 6. For the detection of low amorphous contents, no analytical method exactly fits the optimal content, probably due to imperfections of the mixture. Nevertheless, it is obvious that the NIRS results systematically deviate to higher contents, whereas the XRPD results obtained by the classical evaluation using $I_{net}/I_{obs}$ ratios are too low. The best estimate is given by the chemometric XRPD results, especially by the one that just models the background level. The detection of small crystalline contents is no major problem, as can be seen in Fig. 6. The best estimate is given by the classical evaluation of the XRPD data, i.e., calculating the peak area.

*Table 4.* Estimation of assay errors.

| | XRPD | | | NIRS | |
|---|---|---|---|---|---|
| | sd (%)[a] | sd (%)[c] | sd (%)[c] | sd (%) | n |
| Instrument reproducibility | 0.31 | 0.14 | 0.13 | 0.03 | 10 |
| Sample packing | 0.57 | 0.27 | 0.54 | 0.22 | 10 |
| Day to day reproducibility | 0.44 | 0.3 | 0.18 | 0.37 | 6 |
| Method error (0–10% am.) | 0.38 | 0.3 | 0.2 | 0.3 | 6 |
| Method error (0–10% krist.) | 0.15 | | 0.23 | 0.3 | 3 |

[a]Classical evaluation.
[b]PLSR baseline.
[c]PLSR peaks+baseline.

## Error Estimation

The possible sources of error of XRPD and NIRS are summarized in Table 4. The instrument reproducibility (source and detector variance) and the error induced by different sample packing were determined by measuring the pure crystalline phase in order to avoid errors due to an inhomogenous mixture. The latter was included in the method error. The reproducibility of the NIRS is in general higher than that of XRPD, as effects due to preferred orientation, differing layer thickness of the sample or source, and detector fluctuations were eliminated by MSC and first derivative, whereas the XRPD data weren't preprocessed before evaluation. The normalization technique introduced by Chen, Bates, and Morris[18] could be a solution to further reduce the variability of the XRPD data. The day-to-day reproducibility is higher for XRPD than for NIRS, especially when multivariate calibration was applied.

## CONCLUSION

Our investigation shows that XRPD and NIRS are both perfectly capable of quantifying small amounts of amorphous lactose in a crystalline matrix and vice versa, whereas DSC is not capable of detecting disorders below 10% due to its lack of sensitivity. Moreover, it revealed a nonlinear dependence, probably caused by an incomplete crystallization.

It could be proven that modern XRPD equipments provide detection limits for amorphous material of 0.27% w/w with high accuracy when multivariate calibration (PLS regression) is applied and of 0.93% w/w when simple, univariate calibration is applied. For low crystalline contents the detection limit is about 0.27% w/w for the chemometric approach and 0.45% w/w for the standard integration method. Similar results are obtained by NIRS.

Both methods allow fast and nondestructive measurements, which makes them suitable for routine measurements in pharmaceutical industry.

The possibility of using the calibration curves of XRPD and NIRS for different lactose samples (e.g., different particle size or shape) will be the subject of further studies.

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