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Quantitative determination of crystallinity of alpha-lactose monohydrate by Near Infrared Spectroscopy (NIRS)

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Abstract

The purpose of this study was to determine quantitatively the crystallinity in crystalline/amorphous powder mixtures of lactose, to asses the capability of Near Infrared Spectroscopy (NIRS) for quantitative determination of crystallinity and to compare the accuracy of the NIRS method with that of conventional X-ray powder diffraction (XRPD). Amorphous lactose was prepared by spray drying. Samples with different crystallinity were prepared by physical mixing of 100% amorphous and 100% crystalline materials. The samples were characterized by XRPD and NIRS. Analysis was performed on the data sets by multiple linear regression (MLR). There is a close correlation between the predicted and the actual crystallinity of physical mixtures of crystalline and amorphous lactose, determined by NIRS ($R^2 = 0.9994$). NIRS results were compared to the XRPD using the same sample sets. The correlation coefficients was 0.9981. The results showed that NIRS is an useful method for accurately determining low quantities of the crystalline lactose in a physical mixture. Therefore, NIRS can be used for the quantitative determination of crystallinity of materials during pharmaceutical procedures.

Keywords: Amorphous; Crystalline; Lactose; X-ray powder diffraction; Near Infrared Spectroscopy

1. Introduction

The majority of organic and inorganic solid-state materials in nature as well as most of the synthetically produced materials have crystalline structure. Thus, the majority of solid dosage forms is formulated from crystalline solid raw materials, which have symmetrical crystalline structure. This is a physically and thermodynamically stable state, but some technological operations transform crystalline materials to amorphous form, for example: freeze drying, spray drying, rapid cooling of melt. Undesirably, totally or partially amorphous forms may appear during some manufacturing processes, for example, micronization, strong drying, compaction (Ahlneck and Zografi, 1990). However, amorphous state is a thermodynamically unstable state with higher energy level, so these materials have problems regarding stability and hygroscopicity, resulting in transformation to the more stable crystalline form during storage (Pikal et al., 1978; Saleki-Gerhardt et al., 1994).

Crystalline and amorphous forms of the same material show differences in particle size, particle shape, density, physico-chemical properties, chemical

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stability, water solubility, hygroscopicity, flow properties and compactibility (Nakai et al., 1977; Sund and Grant, 2001). Thus, the particle properties may determine the processibility of materials and the bioavailability of dosage forms (Hancock and Zografi, 1997; Bolhius and Lerk, 1973; Lerk et al., 1974). The amorphous state of a solid powder may alter the bioavailability of a slightly water-soluble drug due to changes in solubility and hence absorption of the drug within the gastrointestinal tract. So, it is very important to know the crystallinity of materials and to monitor it during formulation development, production processes and storage. Determination of crystallinity is of high importance in order to qualify raw materials, intermediates and end products as well as to check the effectiveness of technological procedures during in-process control. Several methods are suitable to study crystallinity such as X-ray powder diffraction (XRPD), density determination, solid state NMR, and water vapour absorption (Buckton and Darcy, 1999). Changes in crystallinity can be detected using DSC and isothermal microcalorimetry techniques, too (Hartauer and Guillory, 1991). Recently, NIRS was introduced as an alternative method for quantification of amorphous and crystalline forms of materials (Hogan and Buckton, 2001).

NIRS has been used extensively in the food and agricultural industries for more than 30 years. In recent years it has become an important analytical technique in the pharmaceutical industry too. NIRS with diffuse reflectance option has many advantages in comparison to other analytical methods because it is a fast and non-invasive technique, requiring no sample preparation or reagents. NIRS collects both chemical and physical information on the material (Plugge and Van der Vlies, 1992). Apart from identification and quantitative analysis, the method can be used to characterize particle size, polymorphism and to control technological procedures, for example, blending, drying, coating (Kamat et al., 1989).

The purpose of this study was to assess the quantitative capability of NIRS for determining crystallinity in crystalline/amorphous powder mixtures of lactose and to compare the accuracy of the NIRS method with that of conventional XRPD. Alpha-lactose monohydrate, as an auxiliary material, was investigated because it is well known that its crystalline and amorphous forms influence the production and suitability of solid-state dosage forms. (Vromans et al., 1987; Shukla and Price, 1991; Riepma et al., 1992). Numerous authors have studied various lactoses by NIRS for identification. However, quantitative determination of crystallinity has not been proven. XRPD is well suited to study changes in the crystalline state but not in the amorphous state. Though it is one of the most widely used techniques, its use is limited due to cost and hazardousness. The detection of crystallinity using XRPD usually is in the range of 5–100%, and sometimes particle size reduction is required.

2. Materials and methods

2.1. Materials

A sample of alpha-lactose monohydrate (Pharmatose DCL 15, DMV, The Netherlands) was used as reference material (referring to 100% crystalline lactose). The particle size was in the range of 50–280 μm. In order to prepare totally amorphous lactose crystalline alpha-lactose monohydrate was dissolved in water at a ratio of 1:10 to obtain a solution for spray drying. Spray-dried (SD) lactose was prepared using an A/S NIRO Atomizer (Copenhagen, Denmark). The processing conditions were as follows: feed rate: 20 ml/min, inlet and outlet temperatures: 175 and 80 °C. The crystallinity of the SD lactose is considered as 0%. The average particle diameter of SD lactose was 5–30 μm. The resulting amorphous particles were kept in a glass vial and stored in a desiccator at 30% relative humidity (RH) and room temperature (50–60% RH is the critical RH for crystallization of amorphous lactose).

Physical mixtures of amorphous and crystalline lactose were prepared to achieve 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 100% crystalline content by weight. The components were weighed to a total amount of 25.00 g and mixed in a Turbula mixer (Turbula WAB, System Schatz, Switzerland) with 10 g of 2 mm glass beads. The powder mixture was stored at 30% RH and room temperature up to the analysis.

2.2. X-ray powder diffraction

XRPD profiles were taken with an X-ray diffractometer (Philips PW 1050/70 PW 1710). The

measurement conditions were as follows: radiation source: Cu Kα, scan speed (2θ): 0.035, step size (2θ): 0.035, time per step (s): 1.0.

2.3. Near Infrared Spectroscopy

The diffusion reflectance was measured by a Hitachi U-3501 UV/Vis/NIR spectrophotometer (Hitachi Ltd., Japan) equipped with integrating sphere ($d = 60$ mm) and PbS detector. Solid samples were placed into the 5-mm layered sample holder of the instrument and the diffuse reflectance spectra were recorded in the 200–2600 nm wavelength range.

3. Results and discussion

3.1. X-ray powder diffraction

Fig. 1 shows the XRPD profiles of the physical mixtures with 5, 50 and 100% crystallinity. The diffractograms of the samples with 5 and 50% crystallinity differ from the diffractogram of the totally crystalline alpha-lactose monohydrate.

The profile of the crystalline form had specific diffraction peaks at 12.39–12.5°; 16.2–16.38°; 19.2° and 19.59°. At these 2θ values the highest relative intensity values can be measured (Table 1). Analysis was performed on these data sets by multiple linear regression (MLR). The dependent variable is the crystallinity and the independent variables are the intensity values at chosen 2θ values. The confidential interval was 95% ($\alpha = 0.05$). From these calculations a calibration equation was achieved:

$$Y = 0.3626 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 \quad (1)$$

Table 1
Intensity values at chosen 2θ values

Crystallinity (%)	Intensity			
	12.39–12.53	16.2–16.38	19.12	19.59
5	100	132	250	350
10	317	441	1243	1457
20	868	711	1905	2298
30	1516	1319	3869	4115
50	1733	1987	4786	5405
70	3042	3295	7604	7604
90	3886	4599	10120	9278
95	3644	4983	10238	9973
100	3901	5174	11638	11131

Table 2
Regression coefficients of Eq. (1), determined by conventional X-ray powder diffraction

2θ (°)	Regression coefficients (a_1–a_4)
12.39–12.53	0.007536
16.2–16.38	0.01496
19.12	−0.00878
19.59	0.008496

where Y is the crystallinity, 0.3626 denotes the intercept, a represents the regression coefficients (Table 2), x denotes the intensity.

On the basis of this equation the predicted crystallinity can be obtained. Fig. 2 shows the relation between the actual and predicted crystallinity. The slope of the line is 0.9978, the intercept is 0.1137 and the correlation coefficient (R^2) 0.9978. Thus, there is a close correlation between the predicted and actual crystallinity values indicating that XRPD is indeed suitable for determining the crystallinity of unknown materials.

3.2. Near Infrared Spectroscopy

Three spectra of each sample were collected and subsequently averaged to produce a single spectrum used for further analysis. The measured reflectance data were transformed into log(1/R) in order to convert reflectance to absorbance.

Comparison of the crystalline and amorphous lactose results in a different absorbance spectrum (Fig. 3), where increased hydrogen bonding shifts OH bands to higher wavelengths (first overtone from 1480 to 1550 nm, second overtone from 1000 to 1060 nm). Apart from different intensities observed in the UV range at 290 and 360 nm, absorbance values of the crystalline form were also lower at the characteristic wavelengths of OH combination band (∼2100 nm) and CH absorption bands at about 1200 nm (second overtone), 1400 nm (combination), and 1760 nm (first overtone).

Quantification of crystallinity was performed using second-derivative spectra (Fig. 4). In order to minimize the well-known effects of particle size and variable scattering of NIR radiation. In addition, the second-derivative spectrum benefits from normalizing the baseline (exclusion of the upward baseline shift) and sharpening spectra features.

Using the second-derivative values, MLR was performed at different wavelengths (Table 3). The wavelengths were chosen on the basis of distinct differences of the spectra. Dependent variable is the crystallinity and the independent variables are the second-derivative values of absorbance values. The confidence interval was set at 95% ($\alpha = 0.05$).

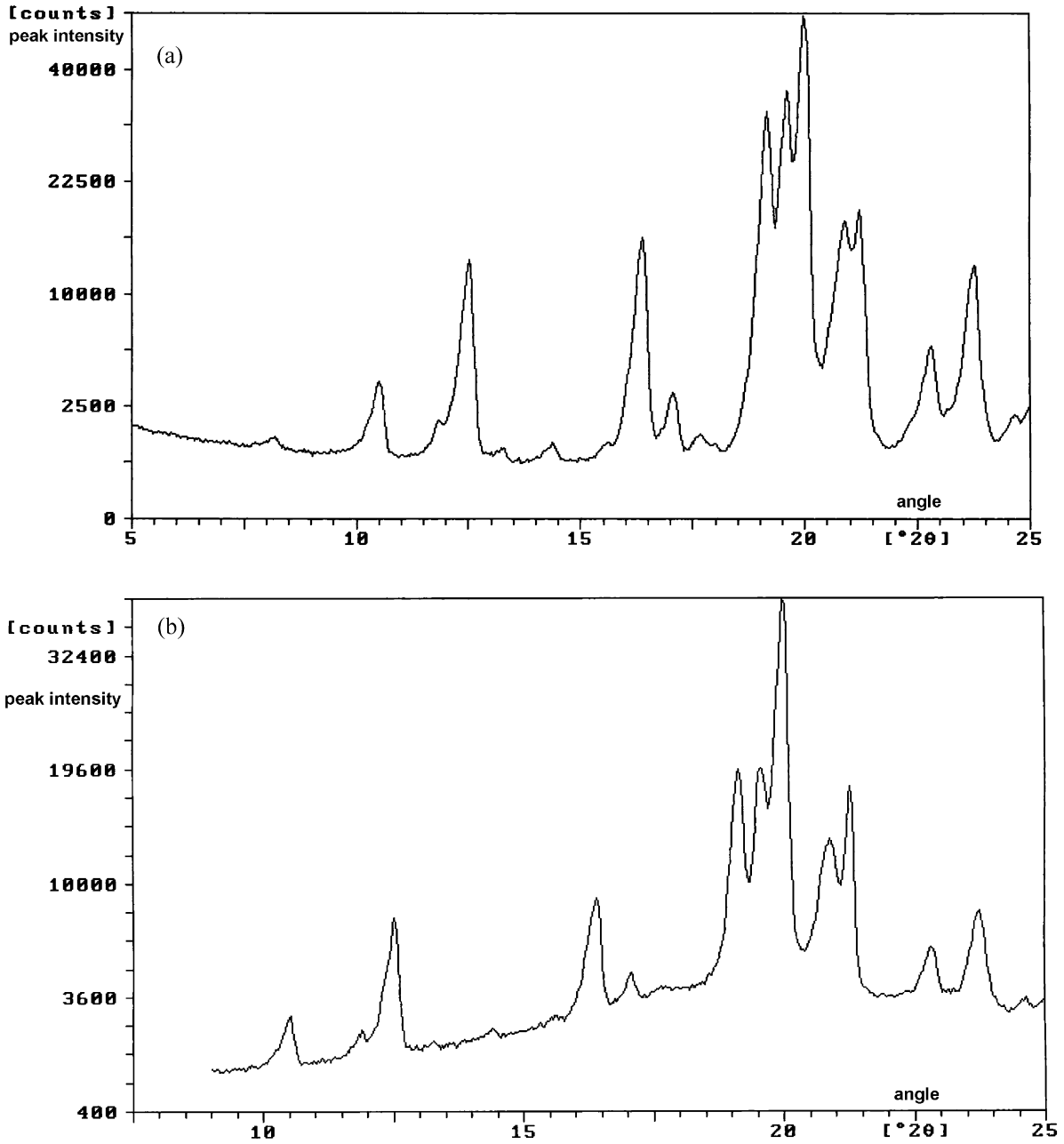

Fig. 1. X-ray diffractograms of the samples with different crystallinities (a: 100% crystallinity; b: 50% crystallinity; c: 5% crystallinity).

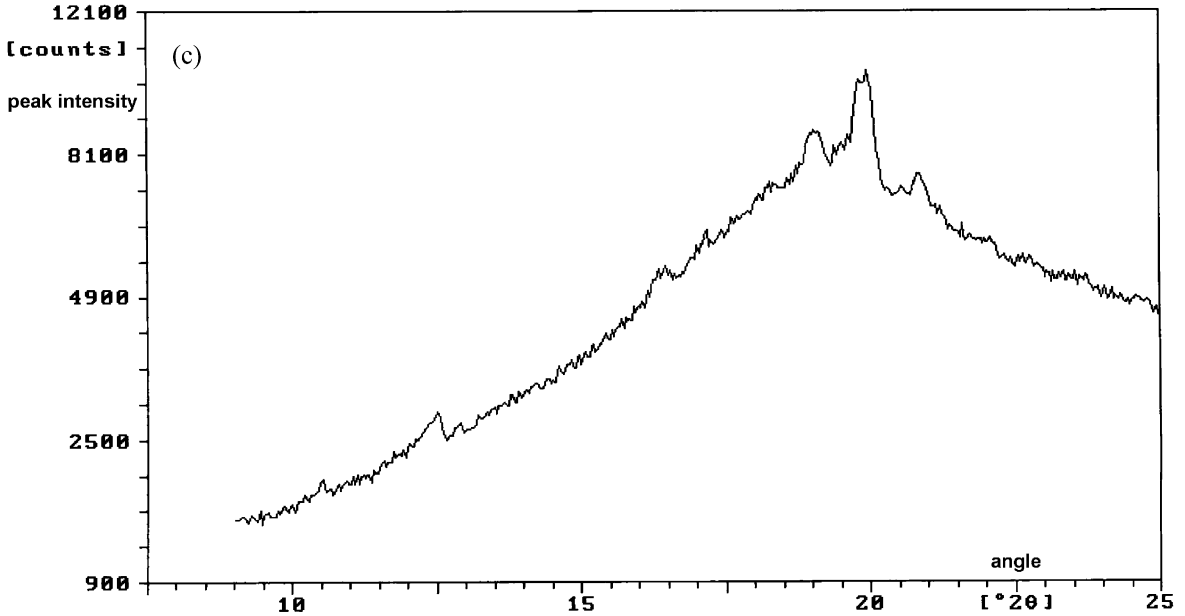

Fig. 1. (*Continued*).

The following calibration equation was constructed:

$$Y = 257.62 + a_1x_1 + a_2x_2 + \cdots a_{11}x_{11}, \quad (2)$$

where Y denotes the crystallinity, the value 257.62 represents the intercept, $a_{1–11}$ denotes the regression coefficients (Table 3), $x_{1–11}$ represent the second-derivative values of absorbance spectra at the characteristic wavelengths.

Fig. 5 shows the relation between the actual and predicted crystallinity determined by NIRS. The value of the correlation coefficient of the determination ($R^2 = 0.9994$) shows a close correlation. Thus, NIR spectroscopy is an appropriate method for the quantitative evaluation of crystallinity of pharmaceutical products.

Finally, NIRS results were compared to the conventional XRPD using the same sample sets (Fig. 6).

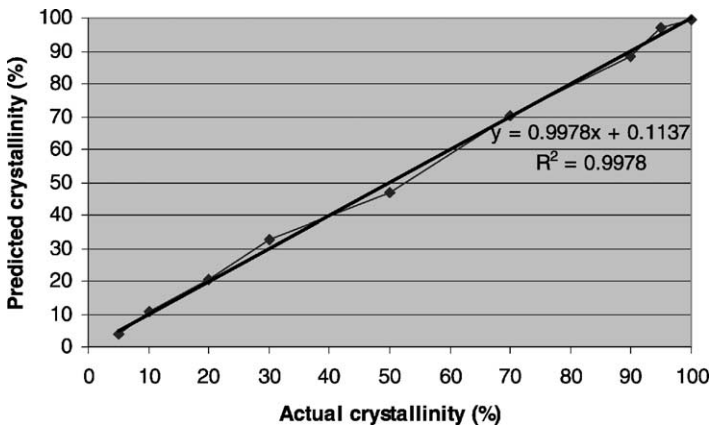

Fig. 2. Relation between predicted and actual crystallinity of physical of physical mixtures of crystalline and amorphous lactose, determined by conventional X-ray powder diffraction.

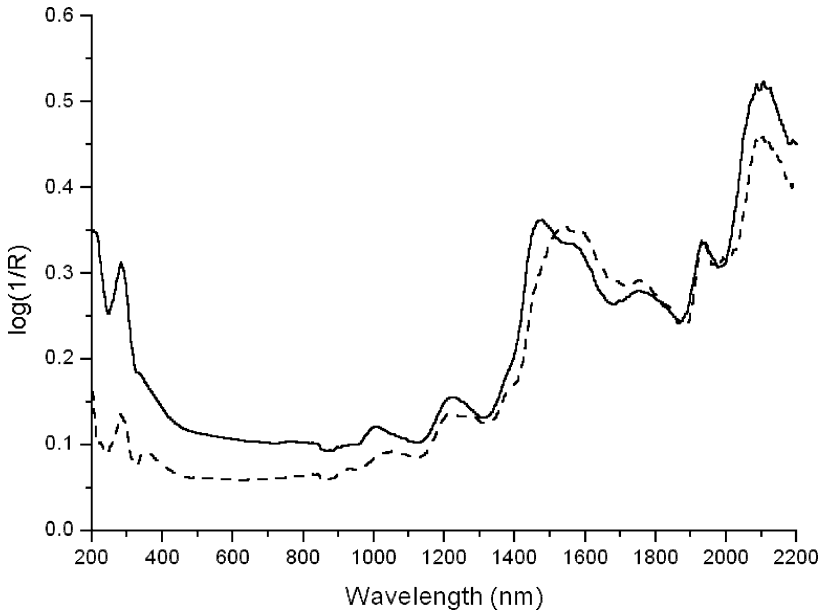

Fig. 3. Absorbance [log(1/*R*)] spectrum of amorphous and crystalline lactose sample (solid: amorphous, dashed: crystalline).

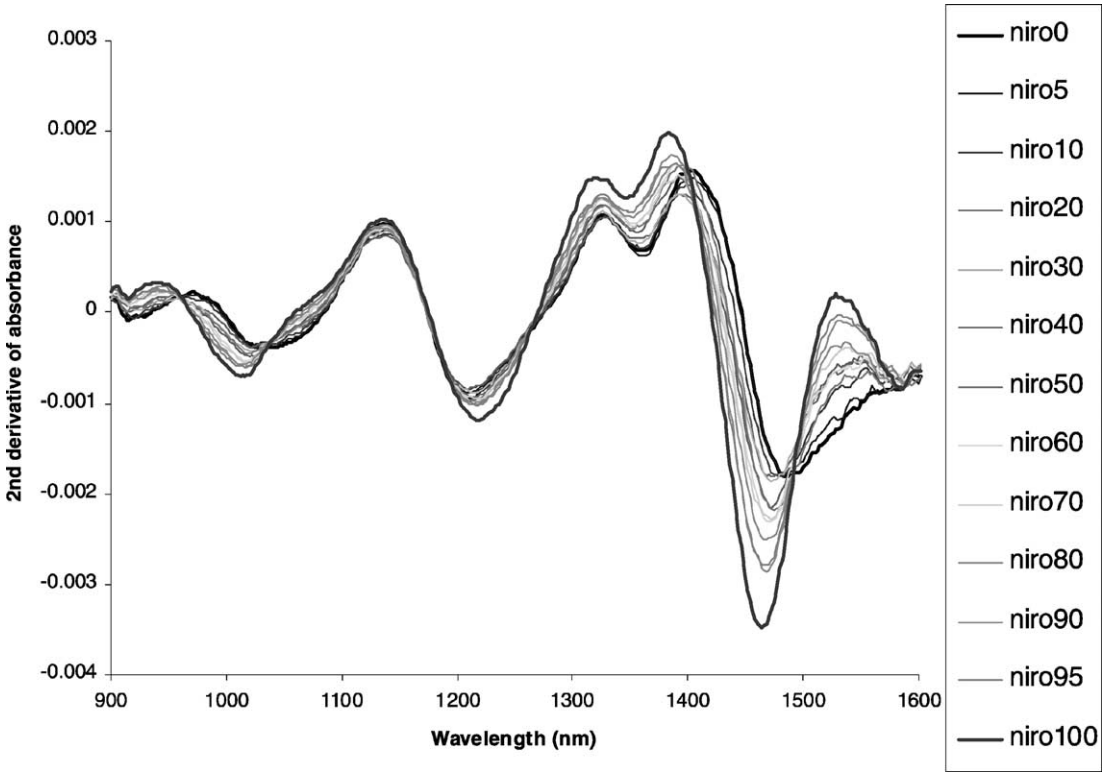

Fig. 4. Second derivative spectra of absorbance spectra of mixtures.

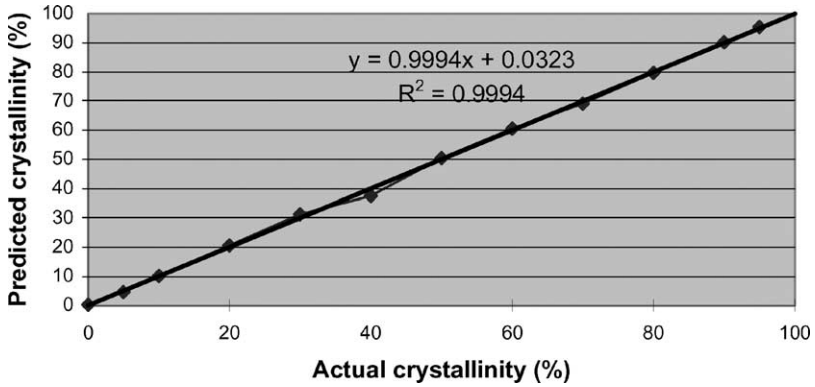

Fig. 5. Relation between predicted and actual crystallinity of physical mixtures of crystalline and amorphous lactose, determined by Near Infrared Spectroscopy.

Table 3
Regression coefficients of Eq. (2), determined by Near Infrared Spectroscopy

Wave number (nm)	Regression coefficients (a_1–a_{11})
298	63.35
386	−1136.86
916	6364.18
1016	−1955.00
1216	−3318.20
1360	−1982.80
1484	1820.77
1534	−44023.55
1536	45880.45
1946	−1442.98
2090	−979.23

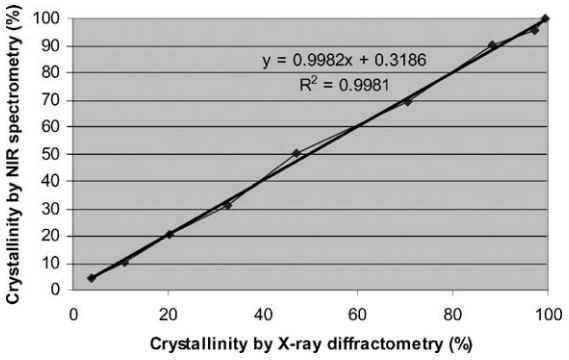

Fig. 6. Relation between predicted crystallinity of lactose mixtures obtained by conventional X-ray powder diffraction and Near Infrared Spectroscopy method.

A straight line can be fitted on the plot with a slope of 0.9982, an intercept of 0.3186 and correlation coefficient (R^2) of 0.9981, indicating that the data generated by XRPD are in a good accordance with the NIRS results.

4. Conclusion

The quality of a pharmaceutical preparation depends on the characteristics of the bulk powders and excipients. Therefore, control of the production processes is very important. The increased use of amorphous drugs in solid dosage forms and the development of amorphous excipients require alternative methods for determination of crystallinity, other than the conventional X-ray diffraction, that are non-destructive, fast, accurate and require minimal sample handling. One suitable method is the NIRS, which is already used for qualitative determination of materials in the pharmaceutical industry. Since particle properties (crystalline or amorphous) may determine the processibility of materials and the bioavailability of dosage forms, it is very important to know the ratio between the crystalline and the amorphous fractions. Moreover, NIRS has the advantage that the identity and crystallinity of the materials can be tested directly in their original containers, omitting sample collection, transportation, storage and identity analysis in the laboratory.

The results of this study revealed, that NIRS is a powerful method for quantitative determination of

crystallinity of alpha-lactose in a physical mixture. Therefore, NIRS can be used for the quantitative determination of crystallinity of materials during pharmaceutical procedures.

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