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Quantitative analysis of amorphous content of lactose using CCD-Raman spectroscopy

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Abstract

A Raman spectroscopy method was developed for the quantification of the amorphous content of lactose. Both physical mixtures and spray-dried samples were used and the results were compared with the IMC determinations. Sample inhomogeneities were averaged out by collecting multiple spectra from each sample, and the total measurement time remained below 10 min due to the high sensitivity of the CCD-Raman spectrometer used in the measurements. The obtained calibration error (SEC) for the physical mixtures was 1.3% (w/w) in the 0–100% amorphous content range and was reduced to 0.2% (w/w) in the 0–10% range of more practical interest. The crystallization heat values of the spray-dried samples showed a linear correlation with the Raman quantifications in the amorphous content range of 0–80%, but saturated over the 80% concentration. This finding suggests a reference value of ca. 60 J/g for the spray-dried samples, instead of the crystallization heat of amorphous lactose (ca. 50 J/g) valid in the IMC determinations of physical mixtures.

Keywords: Raman spectroscopy; Quantification; Amorphous content; Lactose

1. Introduction

Typically, drugs and excipients are highly crystalline materials. It must be noted, however, that they can also contain various amounts of the amorphous phase. The amorphous phase may decrease the physical stability of the compound. On the other hand, the amorphous content may be utilized e.g., to increase the solubility of a poorly soluble material. The methods that are widely used in the quantification of the amorphous content of a sample are X-ray diffraction, moisture sorption and isothermal microcalorimetry (IMC) [1].

In recent years FT-Raman spectroscopy has gained attention as a rapid and non-destructive method of analyzing polymorphic forms of pharmaceutical compounds [1–5]. A small sample volume, determined by the narrow excitation beam of the Raman laser, causes variations between different measurement spots, if the sample is not homogenous. Findley and Bugay [3] have used a Step-n-Repeat sampling accessory to collect multiple spectra from the same sample. This helps to average out sample inhomogeneities, but, at the same time, increases the total measurement time. The number of scans collected for each spectrum was typically 100 [1,4], which means an acquisition time of 10–20 min per spectrum.

In this study a Raman spectroscopy method for the quantification of the amorphous content of lactose was developed, and the obtained values were compared with the IMC determinations. A CCD-Raman spectrometer was used in the measurements, instead of the FT-Raman spectrometers of the previous reports. The change to a more sensitive CCD detector allows shorter measurement times to be used, but the shift of the excitation wavelength from 1064 nm of FT-Raman to, e.g., 785 nm, typical in CCD-Raman, increases the danger

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of fluorescent interference [6]. Fluorescence is not a severe problem, however, with pure pharmaceutical compounds.

2. Materials and methods

2.1. Sample preparation

Lactose was used as a test material, and physical mixtures and spray-dried samples were prepared as described earlier [7].

The physical mixtures were prepared by mixing 100% amorphous lactose and 100% α-lactose monohydrate (Pharmatose® 325M, DMV, The Netherlands). The amorphous content of the mixtures varied from 0 to 100%. The physical mixtures were mixed in a Wig-L-Bug stainless steel mixing capsule without the ball [1]. Mixing (5 min) was done gently in order to avoid deformation of the sample. The particle size distributions of these compounds were close to each other, the $D_{50\%}$ value being ca. 50 μm for monohydrate and 20–40 μm for amorphous lactose (depending on the preparation lot).

The spray-dried samples were prepared with a Büchi Mini-Spray Drier 190 from α-lactose monohydrate [7]. The amorphous content of the samples was controlled by the ratio of ethanol to water in the feed solution. The amorphous content of the samples varied from 0 to 100%. The 100% amorphous lactose used to prepare the physical mixtures was prepared using water as the feed solution.

2.2. Raman spectroscopy

A CCD-Raman spectrometer with a diode laser at the 830 nm wavelength was used in the tests [8]. The laser was focused on the sample through a fiber-optic probe and the scattered power was collected with the same probe. The Raman shift range of the spectrograph was 2000–200 cm^{-1} and its spectral resolution was 8 cm^{-1}. The size of the measurement spot was ca. 0.5 mm (dia.) and the laser power falling on the spot was ca. 100 mW.

A stepping motor was used to implement a multi-point measurement system, which helps to average out the effects of sample inhomogeneities. An acquisition time of only 10 s was needed to obtain a high-quality spectrum for lactose. Six spectra were co-added at each measurement point and seven points were averaged for each sample, resulting a total measurement time of less than 10 min.

2.3. Isothermal microcalorimetry

An isothermal heat-conduction microcalorimeter TAM 2277 was used for determination of the amorphous content [7]. The crystallization temperature and humidity were 25 °C and 54% RH, respectively. The heat accompanying the crystallization was determined in the analysis [7] and a shorter term 'crystallization heat' was used in the text. The analysis time varied between 4 and 16 h, depending on the amorphous content of the sample.

3. Results and discussion

3.1. Evaluation of the Raman method

The Raman spectra of amorphous lactose and α-lactose monohydrate clearly differ from each other over the whole spectral range (Fig. 1). Most bands of these two compounds are, however, overlapping and the largest shifts can be seen in the wavenumber range below 600 cm^{-1}. The bands centered at 470 and 440 cm^{-1} were chosen to represent the monohydrate and amorphous forms, respectively, and a simple band ratio method was used in the calibrations.

The physical mixtures were used to evaluate the calibration method. Because of the large variations in the spectral forms, a non-linear baseline determined by a modified polyfit method [9] was subtracted from each spectrum. The chosen bands evolved smoothly with the changing component ratio (Fig. 2) and the ratio of band areas between 490–455 and 490–410 cm^{-1} for the measurement and reference bands gave almost linear correlation with the amorphous content over the whole concentration range (Fig. 3). A correlation coefficient of $R^2 = 0.991$ with a polynomial fit of second degree was obtained and the corresponding calibration error was only 1.3% (w/w). The samples were homogenous, as indicated by the small value of standard deviation (1.0% (w/w)) (Fig. 3).

Interestingly, when the amorphous content of the physical mixtures was less than 10%, more accurate results could be obtained by simply using a linear baseline (Fig. 4). The linear baseline could be used because the spectral features are very similar for 0–10% amorphous samples. The baseline points

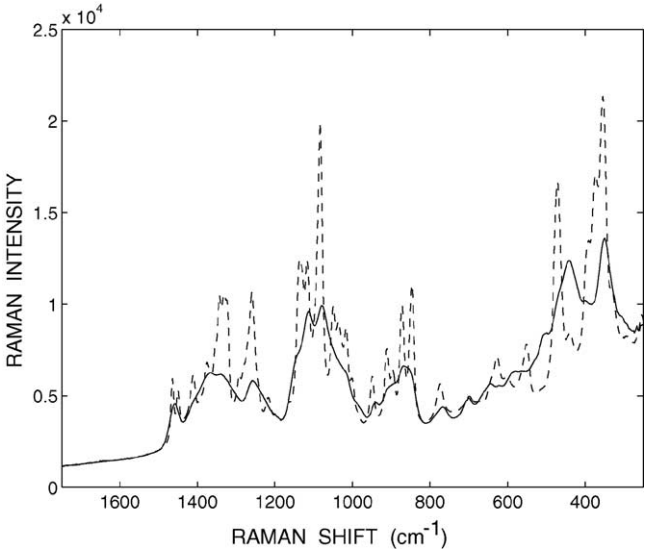

Fig. 1. Raman spectra of α-lactose monohydrate (- - -) and amorphous lactose (—).

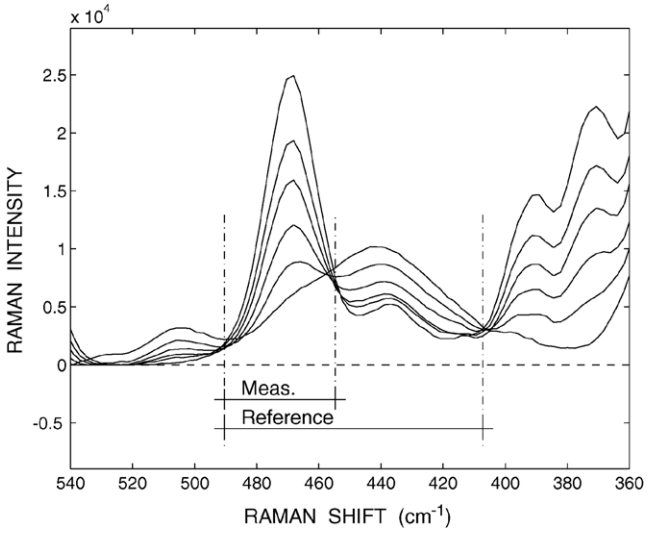

Fig. 2. Spectra of mixed amorphous/monohydrate samples. The band at 470 cm^{-1} increases with increasing monohydrate content: 0, 20, 40, 60, 80 and 100%. Non-linear baselines were subtracted from the spectra and the band areas used in the calibration are shown.

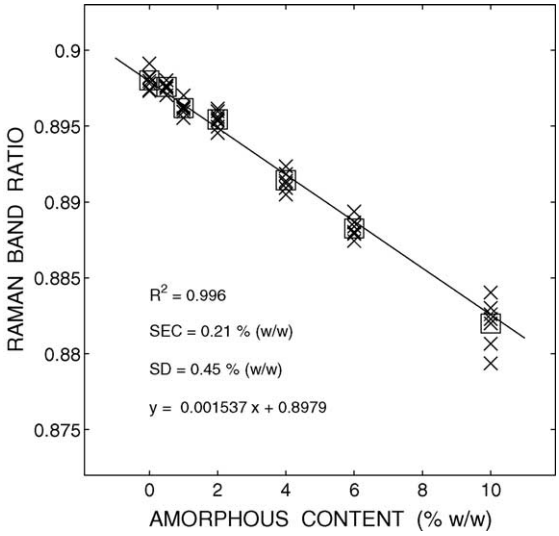

Fig. 4. Band ratio calibration at the low end of the amorphous concentration range. A linear baseline between the wavenumber points 490 and 420 cm^{-1} was used and the reference band was limited between these points. The markers refer to single points (×) and mean values (□).

were chosen at wavenumbers 490 and 420 cm^{-1} (Fig. 2) and the reference band was limited between these points. The measurement band used was the same as before. The correlation of the band ratio with the amorphous content was linear with a correlation coefficient of $R^2 = 0.996$, and the calibration error was as small as 0.2% (w/w) (Fig. 4).

Spectral differences between different polymorphic forms, or between crystalline and amorphous forms, are usually small, and typically the analysis are based on two partially overlapping bands. An algorithm, developed by Kontoyannis et al. [10] and applied by others [1,4], gives a linear correlation over the whole range of component concentrations (0–100%), but is based on the peak height of the bands, and integration of the band areas usually gives smaller variations in the results. We also found that the variations are further reduced if the baseline points are chosen close to the bands, although this baseline does not necessarily represent the zero level of the Raman signal. This was not possible, however, when the whole amorphous/monohydrate concentration range was considered and a new, iterative method for background subtraction developed by Lieber and Mahadevan-Jansen [9] was used.

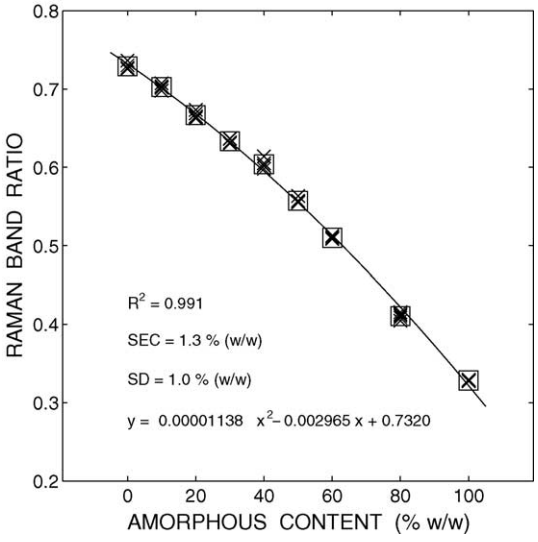

Fig. 3. Ratio of the Raman band areas as a function of the amorphous content in physical mixtures. Correlation was determined for the mean values, and the average variation of separate measurement points is also given. The markers refer to single points (×) and mean values (□).

3.2. Comparison with the IMC method

Two batches of samples were prepared by spray dying. These batches were used to compare the Raman method with the IMC method. Three parallel determinations for each sample were performed with both methods and the mean values were used in the comparison. Average deviations of parallel samples were comparable in both methods, being 1.4% (w/w) for Raman and 1.8% (w/w) for IMC (Fig. 5). The responses determined by the amorphous and monohydrate samples were used in transforming the values into the concentration units.

The calibration function for the Raman quantifications, obtained from the measurements of the physical mixtures, was used when the amorphous content of the spray-dried samples was analyzed by Raman (Fig. 5). This compensates for the non-linearity of the Raman response, which is mainly dependent on the baseline method used and the band areas chosen for the calibration. This kind of calibration transfer is an obvious procedure when homogeneous samples are considered, but in cases of inhomogeneous samples differences

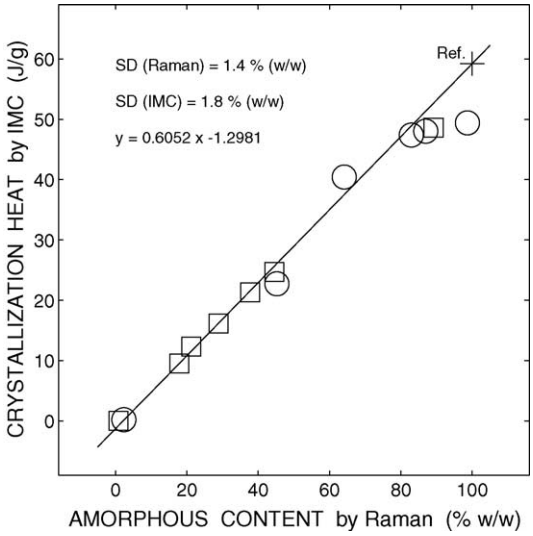

Fig. 5. Comparison of IMC and Raman methods with spray-dried lactose samples. Each point represents a mean value of three parallel samples, and the average variations of both methods are also given. The two markers refer to separate preparation batches and an extrapolated reference point (+) for the IMC determinations is also shown. Only the points below 80% were used when fitting the line.

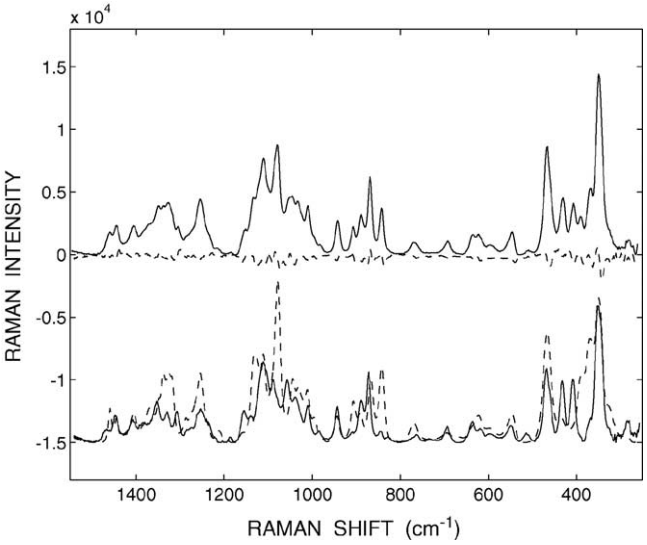

Fig. 6. Spectrum of the crystallized product of amorphous lactose (—/top) compared with α-lactose monohydrate (- - -/bottom) and roller-dried β-lactose anhydrate (—/bottom). Residual of the fitting with a combined spectrum (40/60) is also given (- - -/top).

in scattering properties, which are dependent on particle size and mutual distribution of the components, may cause some uncertainty in Raman quantifications.

The IMC and Raman values show a linear correlation ($R^2 = 0.990$) over the amorphous content range of 0–80%, resulting in a mutual calibration error of ca. 2.0% (w/w) (Fig. 5). When the amorphous content was higher than 80%, the value of the crystallization heat, determined by IMC, did not increase with an increase in the amorphous content (Fig. 5). This result may be related to the finding that in the IMC determinations the crystallized product of amorphous lactose contains some anhydrous β-lactose in addition to the monohydrate [11]. This can be clearly seen in the Raman spectrum of the crystallized product of the 100% amorphous lactose (54% RH, 25 °C). The spectrum can be explained by a combination of the spectra of α-lactose monohydrate and roller-dried β-lactose anhydrate (Fig. 6). The best fit was obtained with a concentration ratio of ca. 40/60 (monohydrate/anhydrate).

The present results suggest that a reference value of ca. 60 J/g ought to be used in IMC determinations of spray-dried lactose samples. This is higher than the crystallization heat of amorphous lactose, ca. 50 J/g, normally used in IMC determinations [11]. The problem is not encountered with physical mixtures of amorphous lactose and lactose monohydrate, because the amorphous fraction crystallizes to the mixture of mono- and anhydrates (40/60), and the response is linear over the whole concentration range.

Combining a Raman spectrometer with an IMC system can be very useful in clarifying the processes that take place during crystallization. The measurement could be performed through the walls of a crystallization ampoule by using a sensitive CCD-Raman spectrometer equipped with a fiber-optic probe.

4. Conclusions

Raman spectroscopy proved to be an effective method in the quantification of the amorphous content of lactose. The calibration error for the physical mixtures containing 100% amorphous lactose and 100% α-lactose monohydrate was 1.3% (w/w) over the whole concentration range and reduced to 0.2% (w/w) in a narrower range of 0–10% amorphous content. In addition to Raman, the amorphous content of the spray-dried lactose samples was determined by IMC. The correlation between the Raman and IMC methods was good, resulting in a mutual calibration error of 2.0% (w/w) over the amorphous content range of 0–80%. When the amorphous content of the sample was higher than 80%, the value of the crystallization heat, determined by IMC, did not increase with an increase in the amorphous content. The result suggests that a higher reference value of ca. 60 J/g ought to be used in IMC determinations of spray-dried samples instead of the crystallization heat of 100% amorphous lactose (ca. 50 J/g) valid for physical mixtures.

Raman spectroscopy has many generic features, which are also advantageous in the determination of the amorphous content of a sample e.g., the method is very fast compared with many traditional methods, usually no sample preparation is needed and the method does not destroy the sample. Errors due to the small sample area, limited by the narrow excitation beam, can be reduced by averaging several measurement points. This can be automated with an accessory driven by a stepping-motor, but the total analysis time tends to increase

to a few hours, when an FT-Raman spectrometer is used. The total analysis time was reduced to less than 10 min by using a more sensitive CCD-Raman spectrometer.

Acknowledgements

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References

[1] L. Taylor, G. Zografi, Pharm. Res. 15 (1998) 755–761.
[2] F.W. Langkilde, J. Sjöblom, L. Tekenbergs-Hjelte, J. Mark, J. Pharm. Biomed. Anal. 15 (1997) 687–696.
[3] W.P. Findlay, D.E. Bugay, J. Pharm. Biomed. Anal. 16 (1998) 921–930.
[4] S.N. Campbell Roberts, A.C. Williams, I.M. Grimsey, S.W. Booth, J. Pharm. Biomed. Anal. 28 (2002) 1135–1147.
[5] N. Al-Zoubi, J.E. Koundourellis, S. Malamataris, J. Pharm. Biomed. Anal. 29 (2002) 459–467.
[6] B. Chase, Appl. Spectrosc. 48 (1994) 14A–19A.
[7] P. Harjunen, V-P. Lehto, J. Välisaari, T. Lankinen, P. Paronen, K. Järvinen, Drug Dev. Ind. Pharm. 28 (2002) 949–955.
[8] P. Niemelä, J. Suhonen, Appl. Spectrosc. 55 (2001) 1337–1340.
[9] C. Lieber, A. Mahadevan-Jansen, Appl. Spectrosc. 57 (2003) 1363–1367.
[10] C.G. Kontoyannis, N.C. Bouropoulos, P.G. Koutsoukis, Appl. Spectrosc. 51 (1997) 64–67.
[11] L.-R. Briggner, G. Buckton, K. Bystrom, P. Darcy, Int. J. Pharm. 105 (1994) 125–135.