ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 687–696

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Quantitative FT-Raman analysis of two crystal forms of a pharmaceutical compound

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Received 25 March 1996; accepted 25 June 1996

Abstract

A pharmaceutical active compound H appears in two polymorphs, A and B, that are stable below and above room temperature, respectively. The A- and B-forms were found to have distinct FT-Raman spectra, in particular for a band at 1716 cm^{-1} (A-form) or 1724 cm^{-1} (B-form). Mixtures of A- in B-form were prepared, and the relative intensity of the characteristic bands at 1716 and 1724 cm^{-1} was found to be proportional to the relative amounts of A- and B-form in the mixtures. A calibration was made which was linear in the range from 1.8 to 15.4% (w/w) of A- in B-form. The results were compared with other methods for analysis of polymorphs: FT-IR spectrometry, differential scanning calorimetry, and powder X-ray diffractometry. A novel FT-Raman sample presentation method for inhomogeneous samples is presented. © 1997 Elsevier Science B.V.

Keywords: Polymorphism; Crystal form; FT-Raman spectrometry; Quantitative analysis; Homogeneity; Pharmaceutical

1. Introduction

Polymorphism is important for pharmaceutical compounds. It may effect their chemical stability, their properties in pharmaceutical formulations, their tendency to absorb water, their solubility, and thus their bioavailability. Consequently, the polymorphism of bioactive materials has been the subject of many studies with different analytical methods. If a large enough single crystal can be obtained, single crystal crystallography is the preferred method. Often, this cannot be done and other methods are used, like powder X-ray diffraction (XRD) [1,2], calorimetry, e.g. differential scanning calorimetry (DSC) [1–5] or thermogravimetry [1], solid state NMR [1,2,6], or Fourier-transform mid-infrared (FT-IR) spectrometry [1–9]. Analysis of organic polymorphs has recently been extensively reviewed by Threlfall [10].

Raman spectrometry has also been used to study polymorphism [2,5,7–11]. Raman spectrometry is not very sensitive to the physical apparition of the sample, which means that many solid samples can be studied directly without sample preparation, but it is sensitive to conditions at

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PII S0731-7085(96)01906-1

the molecular level. Thus, differences are often seen between the Raman spectra from different crystal forms of a compound, or between crystalline and amorphous forms. The possibility of minimal sample preparation and the sensitivity to polymorphism make Raman spectrometry an ideal candidate for studies of crystal forms of pharmaceutical compounds.

Previous studies of polymorphism with Raman spectrometry include a study by Deeley et al. [8] of a compound called R69. This compound has three crystal forms, A, B, and C. Forms A and B were found to have distinct infrared spectra but identical Raman spectra. They were not thought to be separate polymorphs. Form C, a different polymorph, had unique features in both IR and Raman. Quantitative analysis of percentage C-form in mixtures with forms A and B was done with a principal components regression using cross validation. The calibration resulted in a standard error of prediction of 2.33%. Judging from the calibration curve published, the experimental points follow the calibration curve well for high percentages and for ~25, 15, and 5%. For percentages lower than 5, the deviation from the calibration line seems large.

In a Raman study of mixtures of chlorpropamide polymorphs A and B, by Tudor et al. [11], plots of polymorph % (w/w) predicted by principal components regression analysis against polymorph composition determined by weight, showed a linear relationship in the region studied, ca 10–100% (w/w).

When two (or more) enantiotropic polymorphs are present in a sample, one polymorph is stable at a certain temperature and pressure. The other polymorph is unstable and may be present in small amounts. Thus, for a quantitative method to be interesting in industrial applications for the determination of relative amounts of different crystal forms, it must be reliable for low percentages of the minor component.

To this end, we report in the present paper an FT-Raman study of a compound H. H has two enantiotropic crystal forms, A and B. Form A is stable below ~20°C, whereas form B is stable above ~20°C. Conversion from the A- to the B-form is favoured by high temperature and humidity. The thermodynamic equilibrium between the two forms is slow.

In the present work, the A- and B-forms were found to have distinct Raman spectra, and mixtures of the two forms showed relative Raman intensities that were proportional to the amounts of A and B. A calibration curve was constructed which relates the relative spectral intensities to the relative amounts of forms A and B. The quantification of form A was studied with form A as a minor component in form B, in a concentration range as low as we considered practically possible.

Moreover, it is our experience from this and other studies that many samples analyzed show a large degree of inhomogeneity. We found this phenomenon of great importance for the present study, and consequently studied it explicitly. We report a sample presentation method for Raman spectrometry that is well suited to the analysis of inhomogeneous samples.

Comparative studies were done with DSC, FT-IR, and powder XRD, and the advantages and disadvantages of the Raman method relative to powder XRD are discussed.

2. Materials.

H was synthesized and afterwards crystallized using procedures that lead preferentially to either the A- or B-forms. The B-form is stable at room temperature, whereas the A-form was stored in a container with blue gel in a refrigerator when not in use.

To construct a calibration curve, mixtures were produced which consist of known amounts of A-form in B-form. The mixing was done in the following way: 2–9 mg of A and 52–79 mg of B were mixed in an agate mortar with a metal spatula. The major component, the B-form, was added stepwise to A. The mixing was done gently to assure that the polymorphism was not influenced, and for ~10 min in order to be thorough. The B-form was fluffy and sticky, the A-form was harder. Afterwards, the mixture was transferred to a closed stainless steel cylinder placed in a shaker without steel balls (Retsch Vibratory Mill, Type MM-2) and shaken for up to 60 min.

Test spectra were obtained during mixing to study the homogeneity of the mixtures (see below). If a mixture was found to be too inhomogeneous, the mixing was repeated until satisfactory. To produce the lowest concentrations of A studied, additional B was added to mixtures of A in B.

Different batches of H were also investigated. These varied in composition, so that the samples represented both pure B-form and B-form with amounts of A-form varying from ~5 to 30%. Pure A-form had to be crystallized independently.

3. Methods

FT-Raman spectra were obtained on a Perkin Elmer System 2000 FT-Raman spectrometer, equipped with a diode-pumped Nd:YAG laser (1064 nm, 500 mW), a room temperature InGaAs detector, and a quartz beam-splitter. For the quantitative studies, B-stop was: 21.2 mm; J-stop: 3.2 mm at 15 600 cm^{-1}; resolution: 1 cm^{-1}, with 5 data points per cm^{-1}; apodization: filler; OPD velocity: 0.1 cm s^{-1}; phase correction: magnitude; and interferogram type: bi-directional, double-sided. The data region was: 3600–0 cm^{-1} with a filter cut-off at ~200 cm^{-1}. The number of scans per spectrum varied from 32 to 512, highest for low concentrations of A in B.

The samples were put in 5 mm NMR-tubes which were placed vertically in a home-made holder in the FT-Raman instrument. The holder and NMR-tube could be rotated manually around the vertical axis, so that from each sample a number of spectra were obtained with different degrees of rotation. For mixtures this was 0, 45, 90, 135, 180, 225, 270, 315, and 360°. For pure compounds it was 0, 120, and 240°. In this way, different parts of the samples and hence the homogeneity of the samples could be studied, that is the distribution of A- and B-forms. When satisfactory homogeneity was observed, as seen from similarity of spectra, and indicating sufficient amount of mixing, the spectra from one sample were collected and added to give a spectrum with an average distribution of A and B.

For the samples with 15.4, 6.8, 6.4, and 3.1% (w/w) A-form, the holder was replaced by another home-made holder, which was modified so that the NMR-tube was rotated continuously by a motor. This kind of holder seems to be a very important improvement since the spectra automatically average over inhomogeneous samples, and since any tendency towards warming of the samples by the laser light is avoided.

Having finished the present study, we have developed a third version of the holder, where the NMR-tube is rotated continuously by one motor, and moved up and down continuously by another motor. The amplitude in the up-down motion is ~2 mm.

FT-IR spectra were obtained on the same Perkin Elmer instrument, with a TGS detector and a KBr beam-splitter. B-stop was: 21.2 mm; J-stop: 7.8 mm at 5200 cm^{-1}; resolution: 2 cm^{-1}, with 2 data points per cm^{-1}; apodization: strong; OPD velocity: 0.2 cm s^{-1}; phase correction: self; and interferogram type: bi-directional, double-sided. The data region was: 5200–450 cm^{-1}, and the number of scans per spectrum 16. Spectra were obtained in transmission mode. 1.5 mg A-form in 258.4 mg KBr was mixed and mortared gently and afterwards pressed into a tablet, 2 min of vacuum pumping was followed by 2 min at 10 tons pressure on 13 mm diameter disks. For the B-form, 1.4 mg was mixed with 282.8 mg KBr.

In differential scanning calorimetry (DSC), DSC-thermograms were obtained on a Mettler Toledo DSC820 instrument equipped with a sample-robot TSD801RO and controlled by a personal computer with software TA8000. Samples were prepared by weighing 1–5 mg of H into a 40 μl Al-crucible which was sealed with a perforated lid. Experiments were carried out at different heating rates, mostly 10°C min^{-1}, and from 25 to 200 or 250°C in an inert N_2-atmosphere.

4. Results

Many of the spectra used in the present study result after addition or subtraction of experimental spectra. Addition of spectra of course leads to increased absolute intensity. However, we only use the relative intensity in the spectra, i.e. the intensity in one spectrum of bands due to the

A-form relative to bands due to the B-form. When subtracting spectra, the absolute intensity influences the scaling factors in the subtraction, not the relative intensity of bands within the individual spectra.

FT-Raman spectra of the pure A- and B-forms of H in the region 1800–200 cm^{-1} are shown in Fig. 1. The spectra from the two crystal forms differ for many bands. A close inspection of the spectra shows that clear differences (separated bands) are found in the region 1800–1600 cm^{-1}.

This region is shown in detail in Fig. 2, where FT-Raman and FT-IR spectra of both forms are shown together. A couple of interesting features can be seen from the figure. The two forms, A and B, differ clearly in the FT-Raman spectra; the A-form has a peak maximum at 1716 cm^{-1}, the B-form a peak maximum at 1724 cm^{-1}. They also differ in the FT-IR spectra, but each form has more bands than for FT-Raman, and the individual bands are broader in FT-IR, although the FT-Raman and FT-IR spectra in Fig. 2 all were recorded with 2 cm^{-1} resolution.

All in all, in FT-Raman the bands from A and B are nearly base-line separated, which is definitely not the case in FT-IR with broad overlapping bands. Smaller bandwidth in FT-Raman than in FT-IR is often observed and is important for analytical applications of the two methods.

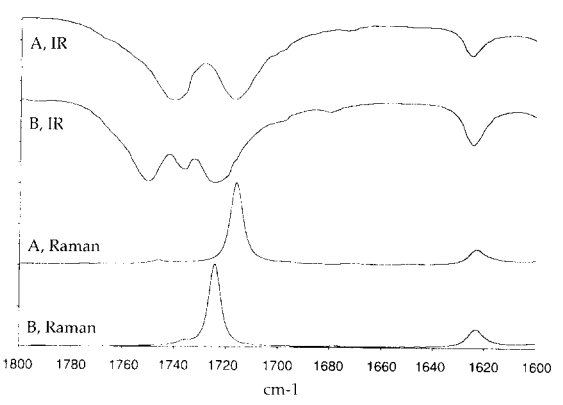

Fig. 2. Comparison of band shape in FT-IR and FT-Raman spectra of the A- and B-forms of H in the region 1800–1600 cm^{-1}. FT-Raman (1064 nm): pure compounds in NMR-tubes. FT-IR (transmission): ~0.5% (w/w) A or B in pressed KBr tablets. Ordinate: The FT-IR spectra have transmission minima of 15% in the region shown, Raman intensity 150, spectra are offset. Resolution: 2 cm^{-1}.

In Fig. 3 are displayed FT-Raman spectra of pure A, pure B, and a mixture of 5.5% A in B (w/w). It is seen that even for a concentration as high as 5.5%, the A-form appears only as a shoulder on the B-form band. This effect was much more pronounced with a resolution of 4 cm^{-1}. Clear differences in spectral band resolution were seen between instrument resolutions of 4, 2, and 1 cm^{-1}. It was found advantageous to use an instrument resolution of 1 cm^{-1}, although it means

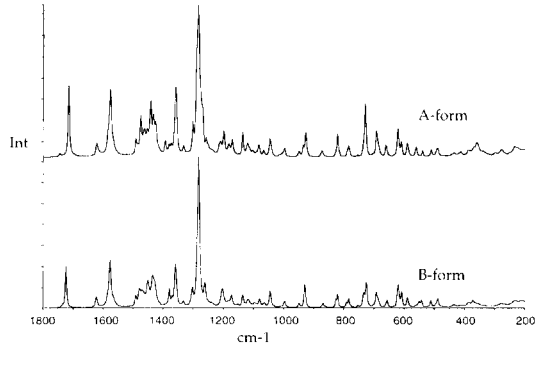

Fig. 1. FT-Raman spectra (1064 nm excitation) of the A- and B-forms of H in the region 1800–200 cm^{-1}. Raman intensity 220 (form A) and 180 (form B), spectra are offset relative to each other. Pure compounds in 5 mm NMR-tubes, resolution 2 cm^{-1}.

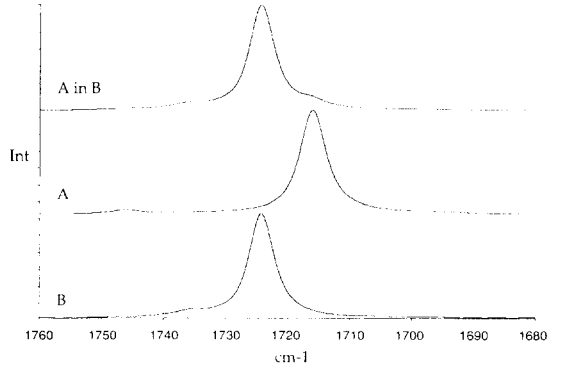

Fig. 3. FT-Raman spectra of pure A-form, pure B-form, and a mixture of 5.5% (w/w) A in B. Raman intensity: 240 (A-form), 150 (B-form), and 636 (mixture, sum of 9 spectra), spectra are offset. Abscissa: 1760–1680 cm^{-1}, resolution: 1 cm^{-1}.

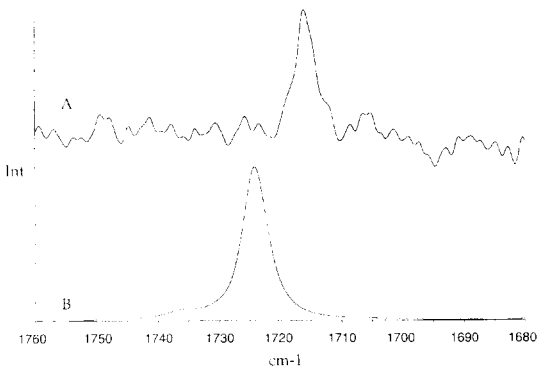

Fig. 4. FT-Raman spectra of contributions from A- and B-forms to the spectrum of a mixture of 1.8% (w/w) A in B. Spectrum 4A results after spectral subtraction of pure B from the mixture; spectrum 4B results after spectral subtraction of pure A from the mixture. Raman intensity: 10 (A-form) and 400 (B-form), spectra are offset. Abscissa: 1760–1680 cm^{-1}, resolution: 1 cm^{-1}.

a large increase in scan time. But still, subtraction procedures seem necessary to separate the contributions from the A- and B-forms to the spectra of mixtures.

The subtraction procedure is illustrated in Fig. 4, which is based on a spectrum from a mixture of 1.8% A in B. Subtraction from the 1.8% mixture spectrum of the spectrum of pure B results in the contribution from pure A, spectrum 4A, to the mixture spectrum. Subtraction from the mixture spectrum of the spectrum of pure A results in the contribution from pure B, spectrum 4B, to the mixture spectrum. Both resulting spectra are seen to have an acceptable S/N-ratio, indicating that detection of as little as 1.8% of one crystal form in another is possible.

Similar subtractions were done for relative amounts of A-form in B-form: 1.8, 3.1, 5.5, 6.4, 6.8, 9.5, 10.4, and 15.4%. A calibration curve is displayed in Fig. 5. The abscissa values are known relative amounts of A in B, whereas the ordinate values correspond to the intensity of the 1716 cm^{-1} band (characteristic for A) relative to the intensity of the 1724 cm^{-1} band (characteristic for B). The data points are quite close to a straight line, and a regression line is added to the figure. The equation for the regression line is as follows, with X = relative amount of A/B (%, w/w), and Y = relative FT-Raman intensity (%, 1716 cm^{-1}/1724 cm^{-1}):

$$Y = 0.99 \times X + 0.27$$

For the mixtures of 10.4, 5.5, and 1.8% A in B, a number of data points are shown in Fig. 5. These correspond to the individual spectra from different degrees of rotation of the NMR-tube and show the spread in the Raman values due to inhomogeneity of the sample. The spread is similar for the three mixtures investigated in this manner.

The calibration curve in Fig. 5 does not pass through origo, which it should according to theory. The reason for this must be found in experimental uncertainty, in the estimation of band intensities, but mostly in imperfect sampling over inhomogeneous samples.

The calibration model was tested on two other samples. One sample contained little A-form, the other sample contained a large amount of A-form. Subtraction from the mixture spectra of spectra of pure A- and B-form, respectively, gave the spectral residuals from B- and A-form in the samples. The relative intensities of the 1716 and 1724 cm^{-1} peaks were: 5.0 and 37.9% for the two samples, leading to relative percentages of 4.8 and 38 of A in B. Of these, 38% is actually extrapolated outside the range of the present method, see below.

The amount of A in these two samples was estimated from powder XRD measurements. For the sample with the high amount of A, this gave 30% of A, whereas for the sample with lower amount of A, 6% of A was found from powder XRD. The XRD value of 30% indicates that the Raman value of 38% is reasonable, even though it is outside the calibration range. The Raman value may be more dependable, since the XRD measurement did not take into account the rather large inhomogeneity observed with Raman for this sample. For the other sample, the discrepancy between the Raman value of 4.8% and the XRD value of 6% is small, and here the XRD value is somewhat uncertain due to a low signal-to-noise ratio.

The spectra for these two samples each resulted from addition of several spectra from the same sample with rotation of the NMR-tube in between

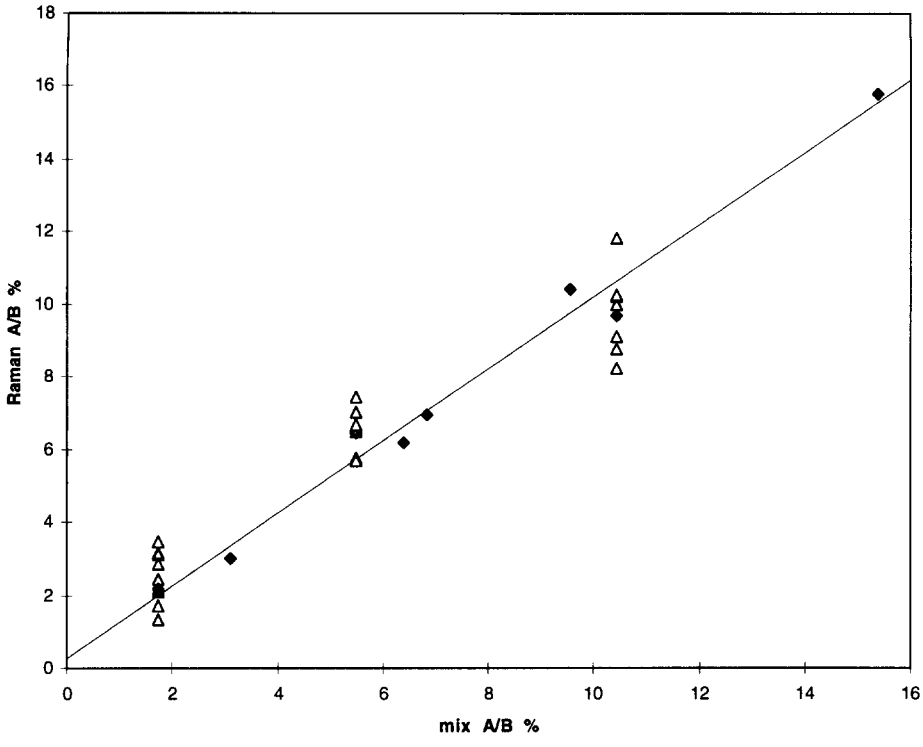

Fig. 5. Calibration curve between relative Raman intensities and relative amounts of A and B. Abscissa: relative amount A/B (%, w/w). Ordinate: Raman intensity at 1716 cm^{-1} (A-form) divided by intensity at 1724 cm^{-1} (B-form), in %. (◆): Data from sum of spectra with different angles of rotation. (△): Data from spectra with different angles of rotation for the mixtures with 10.4, 5.5, and 1.8% A in B. Solid line is a least-squares regression line.

the spectra. For the two samples, a large variation was observed between different spectra from the same sample, showing that these samples were quite inhomogeneous. The distribution of A-form was more inhomogeneous over these 'real' samples than for the samples mixed for the present study.

An alternative determination of the A- and B-forms was done with differential scanning calorimetry (DSC). The result of this study is shown in Fig. 6. The DSC curve for pure B-form (curve 1) shows a clean melting behaviour with an endotherm at 144°C. For the pure A-form (curve 4), the strong endotherm at 130°C corresponds to melting of the A-form. However, this endotherm is followed immediately by a small exotherm which probably corresponds to a resolidification to B-form. This is confirmed by the observation of a melting endotherm around 142°C, characteristic for the B-form. The fact that the A-form did not contain any B-form before the DSC measurement was confirmed by FT-Raman. Thus, the resolidification to B-form takes place without any seed of B-form. A similar behaviour is observed for the curves for the samples with 4.8% (curve 2) and 38% (curve 3) A in B.

It is typical for a low melting polymorph, which is free from seeds of a higher melting polymorph, to overshoot and melt (endotherm) at its own melting point, often followed by resolidification (exotherm) to the higher melting polymorph [10].

An additional problem in connection with DSC was the inhomogeneity in the distribution of the A-form over the samples. A proper determination of relative amounts of A and B would involve DSC measurements on several capsules from a certain batch to get representative results.

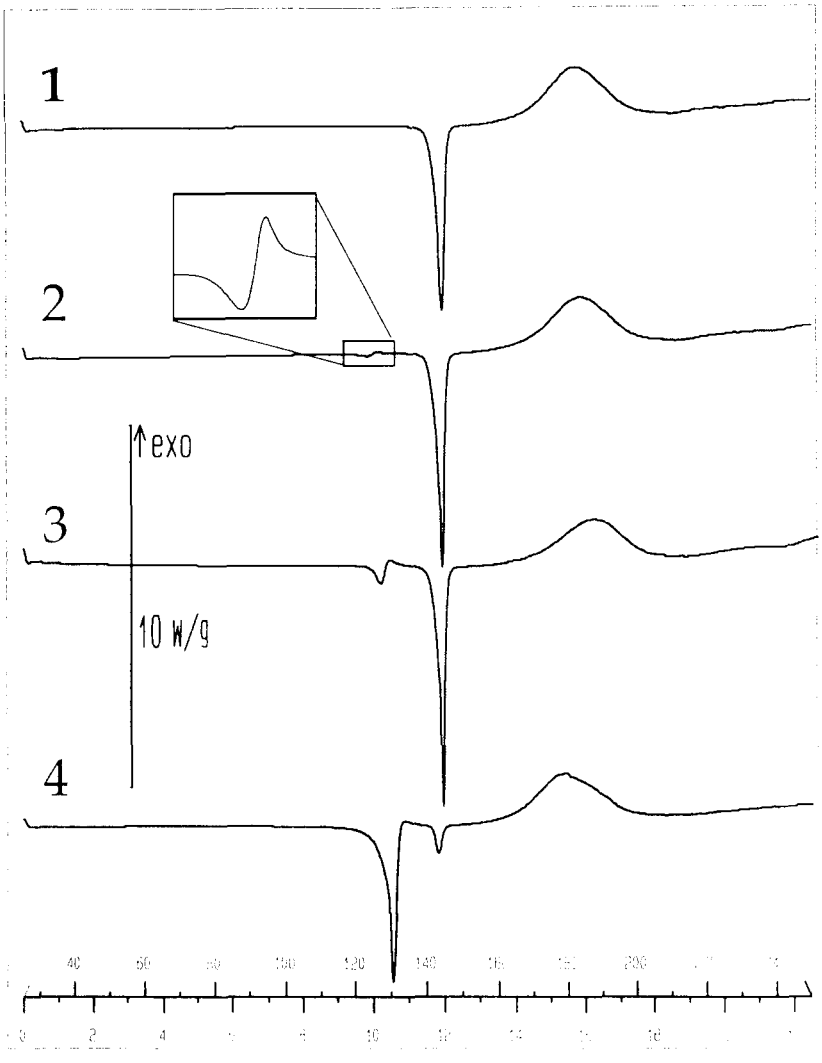

Fig. 6. Differential scanning calorimetry (DSC) thermograms in the range 25–250°C, 10°C min^{-1}. Curve 1 (top): Pure B-form, melting endotherm at 144°C. Curves 2 and 3: ~4.8 and 38% A- in B-form, respectively. Curve 4 (bottom): Pure A-form, melting endotherm at 130°C, followed by resolidification to B-form with melting endotherm at 142°C. Exotherms at 180°C correspond to degradation of the substance. Abscissa: temperature in °C and time in minutes. Ordinate: Effect in Watt/gram.

5. Discussion

We shall first discuss the sample presentation with the sample held in a rotating NMR-tube. During the present work, a report appeared by Kaminaka and Kitagawa [12], describing a sample holder rotating a 5 mm quartz tube, for UV resonance Raman (UVRR) measurements of powder and small-volume solution samples in back-scattering geometry.

In the present study, we independently developed a holder very similar to the UVRR one described in [12]. The UVRR holder was designed to avoid sample heating in UVRR spectroscopy. The holder from the present study was designed with three purposes: (1) to provide a practical

container for powders instead of the open metal rings, standard in FT-Raman, where the sample is fixed by pressing it hard into the ring, we wanted to avoid pressing the sample when studying polymorphism; (2) to avoid laser heating of the sample by rotating the sample; and, most importantly, (3) to increase the amount of sample studied to compensate for inhomogeneities in the sample, that is to avoid sub-sampling.

By rotation of the sample, the volume studied increases dramatically. In our instrument, we regard the cone shaped volume hit by the laser using a stationary sample holder as having a base diameter of ~0.4 mm and a depth of ~1 mm, depending on the depth of penetration of the laser light. This cone corresponds to a volume of 0.04 mm^3. With rotation, the cone is replaced by a ring with an outer diameter of ~4 mm and an inner diameter of ~2 mm. The height of the ring at the glass wall of the tube is ~0.4 mm, decreasing to zero 1 mm into the sample. We estimate the volume of this ring to be ~2 mm^3, which is 50 times larger than that of the cone. With the latest version of this holder where an up-down motion is added, see above, we estimate the volume studied as ~10 mm^3.

With stepwise rotation it is possible to study the degree of homogeneity of the sample, whereas with continuous rotation a spectrum is obtained representing the average distribution of species within the ring-shaped volume studied. One might expect band-broadening or side-band effects when studying a rotating sample in FT-Raman. However, with the spinning rates used here, ~10–20 rounds per min, no such effects were observed.

Turning now to the results relating to the polymorphism of the compound H, we observe several important points:

(1) It is possible with FT-Raman spectrometry to clearly distinguish between the crystal forms A and B of compound H. The clearest difference is seen for the bands at 1724 and 1716 cm^{-1}. These bands are in a region characteristic of conjugated esters, 1735–1715 cm^{-1}, [13]. This suggests that a conjugated ester moiety of the molecule is an important factor for the packing of the molecules in the crystal structures of the two polymorphs of compound H.

(2) The relative signal intensity of A- to B-form is quantitatively proportional to the relative amount of A-form to B-form. The relationship can be expressed in a linear calibration curve.

(3) The lower limit of concentration studied in the present work was 1.8% (w/w) A- in B-form. This concentration was limited by the difficulty in mixing the two crystal forms. The signal-to-noise ratio for the band from the A-form after subtraction in the experiment with 1.8% A was ~5 (Fig. 4). Thus, the method can be used down to relative concentrations of 1% of the minor crystal form. With longer scan time, this limit could be further reduced.

(4) The calibration curve is linear up to the data set with the highest amount of A: 15.4%. The method is thus applicable in the range 1–15% of the minor component.

(5) An additional result of the present method is that it yields information on the degree of homogeneity in the distribution of A and B over the sample.

Some points of caution have to be kept in mind with a method like the present one. The absolute intensity of the Raman signal is dependent on particle size. There has been confusion in the literature about the nature of the relationship, but a recent study shows Raman intensity growing with decreasing particle size [14]. So when methods like the present one are used on real production batches one must assure that the variation in particle size is limited, or at least that the variation in particle size is similar for the different components in the mixture.

Another point of caution is the heating of the sample which can take place even with a laser wavelength of 1064 nm. In the present study, heating effects were studied by repetition of spectra from the same small volume of a sample, and by comparison between spectra obtained with stepwise and continuous rotation of the sample. Any heating effects were below the limit of detection.

We would like to comment that for many practical applications of Raman spectrometry it is preferable to use an instrument where an acceptable signal intensity is obtained with a laser focus that is not very tight, in order to avoid sub-sampling.

In the evaluation of the present results, one must compare with alternative methods. Here, we shall mention FT-IR spectrometry, powder X-ray diffraction (XRD), and differential scanning calorimetry (DSC).

Comparison between FT-IR and FT-Raman spectrometry can be made from Fig. 2. It is seen from the figure that in the FT-IR spectra clear differences also appear between the A- and B-forms. However, the bands in FT-IR are broader and overlap more, so the possibility to distinguish between the different crystal forms is larger for FT-Raman than for FT-IR. Moreover, the FT-Raman spectra were obtained directly from the neat compounds, whereas the FT-IR spectra demand sample preparation including mortaring to ensure small and homogeneous particle size. This grinding may influence the crystal modification, a fact that is clearly disadvantageous.

We cannot rule out that some change in crystal modification results from the grinding and pressing, but the difference between the FT-IR spectra of forms A and B does not point in this direction.

The necessity of sample preparation also applies to FT-IR measured in diffuse reflectance, but with photoacoustic detection (PAS) FT-IR spectra can be obtained directly from the compounds. Thus a more just comparison could have been between FT-Raman and PAS.

One further perspective in the use of FT-Raman spectroscopy for analysis of polymorphism is connected with good possibilities to use FT-Raman in situ. Thus, FT-Raman spectra can be obtained from whole, undisturbed, pharmaceutical tablets, and it is possible to study directly in a pharmaceutical formulation whether the active ingredient has one or another crystal form, or is amorphous. Such changes in the crystal form might take place with high temperature and pressure during compression of a tablet.

Powder X-ray diffraction of crystalline compounds yields diffractograms that are significant for the individual crystal forms. If an XRD diffractogram is obtained from a mixture with known amounts of different crystal forms, a calibration curve can be constructed between relative amount of the crystal form in question and the relative intensity of the XRD bands belonging to that crystal form, in the same way as has been done in the present study with FT-Raman spectrometry.

However, XRD and FT-Raman spectrometry differ when amorphous phases are present. For the amorphous phase, XRD does not give clear peaks, but only a broad structureless feature. In this situation, FT-Raman has a clear advantage, since in FT-Raman the amorphous phase gives specific bands, very much like the ones from crystalline phases, but generally broader. Mostly, there is even a shift in FT-Raman spectra between crystalline and amorphous phase, not so much in frequency as in relative intensity.

For comparison with DSC, we refer to the results above. DSC yields signals corresponding among others to melting of one or more crystal forms and to transitions between the forms. The signals relate quantitatively to the amount of heat necessary to induce the transitions. In the present case it was found that for the melting of the A-form a quantitative interpretation of the signal was made more difficult by the concomitant transition to the B-form.

6. Conclusions

In the present study, we have observed different FT-Raman spectra from the two polymorphs A and B of compound H, and identified a frequency shift that leads to two well-resolved bands. We found that for mixtures of A in B, the intensity of the two bands is proportional to the amount of A and B forms. We constructed a calibration curve that was linear in the region 1.8–15.4% (w/w) of A in B.

We described a novel sample holder for FT-Raman spectrometry where the sample is held in an NMR tube which is rotated around its axis and at the same time moved up and down. This sample presentation leads to a large increase in the volume studied. This is important for inhomogeneous samples where sub-sampling is a problem. Moreover, possible degradation of the sample through heating by the laser is avoided.

The present results from FT-Raman work were compared with results from FT-IR and differen-

tial scanning calorimetry, and the relationship to powder X-ray diffraction was discussed.

Finally, the calibration in the present work was based on the intensity at only one wavelength for each of the two crystal forms. This procedure works for compounds with clear separation between peaks. However, it is generally preferable to use the whole spectrum for the calibration, in particular when an amorphous phase is present. This can be done by analyzing the spectra with chemometrics programs, using procedures like principal components analysis (PCA), principal components regression (PCR), or partial least squares (PLS). We have made a preliminary chemometric study of the present data, it seems to work well, and shall be reported at a later stage.

References

[1] H.G. Brittain, K.R. Morris, D.E. Bugay, A.B. Thakur and A.T.M. Serajuddin, J. Pharm. Biomed. Anal., 11 (1993) 1063–1069.

[2] A. Terol, G. Cassanas, J. Nurit, B. Pauvert, A. Bouassab, J. Rambaud and P. Chevallet, J. Pharm. Sci., 83 (1994) 1437–1442.

[3] M. Kuhnert-Brandstätter and H.W. Sollinger, Mikrokhim. Acta, III (1990) 233–245.

[4] M. Kuhnert-Brandstätter and H.W. Sollinger, Mikrochim. Acta, III (1990) 247–258.

[5] M. Kobayashi and T. Adachi, J. Phys. Chem., 99 (1995) 4609–4619.

[6] R.A. Fletton, R.K. Harris, A.M. Kenwright, R.W. Lancaster, K.J. Packer and N. Sheppard, Spectrochim. Acta, 43A, (1987) 1111–1120.

[7] A.M. Tudor, M.C. Davies, C.D. Melia, D.C. Lee, R.C. Mitchell, P.J. Hendra and S.J. Church, Spectrochim. Acta, 47A (1991) 1389–1393.

[8] C.M. Deeley, R.A. Spragg and T.L. Threlfall, Spectrochim. Acta, 47A, (1991) 1217–1223.

[9] G.H. Neville, H.D. Beckstead and H.F. Shurvell, J. Pharm. Sci., 81, (1992) 1141–1146.

[10] T.L. Threlfall, Analyst 120, (1995) 2435–2460.

[11] A.M. Tudor, S.J. Church, P.J. Hendra, M.C. Davies and C.D. Melia, Pharm. Res., 10, (1993) 1772–1776.

[12] S. Kaminaka and T. Kitagawa, Appl. Spectrosc., 49, (1995) 685–687.

[13] D. Lin-Vien, N.B. Colthup, W.G. Fateley and J.G. Grasselli, The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules, Academic Press, New York, 1991.

[14] M.V. Pellow-Jarman, P.J. Hendra and R.J. Lehnert, The dependence of Raman signal intensity on particle size for crystal powders, (1996) submitted for publication.