International Journal of Pharmaceutics 394 (2010) 18–25

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Quantitation of a polymorphic mixture of an active pharmaceutical ingredient with solid state ^{13}C CPMAS NMR spectroscopy

Tommi Virtanen*, Sirkka L. Maunu

Department of Chemistry, Laboratory of Polymer Chemistry, A. I. Virtasen aukio 1, P.O. Box 55, 00014 University of Helsinki, Finland

ARTICLE INFO

Article history:
Received 17 November 2009
Received in revised form 5 March 2010
Accepted 13 April 2010
Available online 21 April 2010

Keywords:
Solid state NMR
Polymorphism
Quantitation
DECRA

ABSTRACT

Active pharmaceutical ingredients (API) often crystallise in several forms with significant differences in their physical properties. In pharmaceutical applications it is very important to be able to identify these polymorphs during drug manufacturing and storaging, also quantitative information about polymorphs is often required. Solid state ^{13}C cross-polarisation (CP), magic angle spinning (MAS), nuclear magnetic resonance (NMR) spectroscopy was utilised in studying polymorphisity of an API with two polymorphic forms. Quantitative information was obtained from polymorphic mixtures, and a formulated product was also studied in order to determine the possibility of distinguishing between the two polymorphs in a low-dose formulation. Quantitative data was obtained using two methods: integration of signals from the dipolar dephased spectra, and a chemometric method known as Direct Exponential Curve Resolution Algorithm (DECRA). We concluded that the two polymorphs are easily identifiable based on their spectral differences. Quantitative results showed reasonable accuracy, and while identification of the polymorph present in formulation was not possible, traces of the API are detectable in as low dosage as 0.7% by weight using solid state NMR methods.

1. Introduction

Small organic molecules typically exhibit polymorphism, *i.e.* they can crystallise in more than one form. These polymorphs may show notable variation in their physical properties, including stability and processability. For pharmaceutical applications this is of great concern. Knowledge of the precise crystalline form of an active pharmaceutical ingredient (API) at all stages of the drug manufacturing process and the effects of storage on the formulated product are extremely important. Although high stability of the API may be desirable at the production stages, it may cause the drug not to be soluble in the blood circulation, and therefore remain inactive. A conversion from one polymorphic form to another more stable one during storage may therefore have unfavourable effects for the end user of the drug.

Polymorphism of pharmaceutical compounds has been studied by a variety of methods (Bugay, 2001; Harris, 2006; Offerdahl, 2006; Brittain et al., 1991; Stephenson et al., 2001) including X-ray diffraction (XRD) and X-ray powder diffraction (XRPD) (Roberts et al., 2002; Agatonovic-Kustrin et al., 2001; Kordikowski et al., 2001; Raghavan et al., 1993), differential scanning calorimetry (DSC) (Sheikhzadeh et al., 2006), and infra-red (IR) and Raman spectroscopies (Patel et al., 2000; Gamberini et al., 2006; McMahon et al., 1996). Of these methods, X-ray diffraction is the most common tool for obtaining information about crystalline structures. Solid state ^{13}C cross-polarisation magic angle spinning (CPMAS), nuclear magnetic resonance (NMR) spectroscopy has also become a standard method for analytical work in pharmaceutics, and quite many studies have used ^{13}C CPMAS NMR to study APIs, from basic characterisation to more advanced experiments, often combined with computational work and other spectroscopic methods (Smith et al., 2006; Tozuka et al., 2002; Gervais et al., 2004; Yates et al., 2005). The ^{13}C solid state NMR spectrum of a sample can be considered a fingerprint of that material. Structural and chemical changes of the material can readily be observed by simple inspection of the spectrum due to sensitivity of the chemical shift values to alterations in local environments of the nuclei. This makes solid state NMR spectroscopy an excellent tool for studying polymorphism. One benefit of ^{13}C CPMAS NMR spectroscopy is that the sample does not need to be a single crystal. A high quality spectrum can also be obtained from powder samples, and amorphous materials can be studied as well (Gustafsson et al., 1998). Long measurement times, in some cases several days, are a drawback of solid state NMR spectroscopy.

Although NMR spectroscopy is an inherently quantitative method, the signal intensity from a given site is proportional to the number of those sites in sample, several experimental aspects need to be considered when measuring quantitative ^{13}C spectra. While this is also true for liquid state NMR spectroscopy, it becomes essential with solid state NMR spectroscopy (Harris, 1985). The

* Corresponding author. Tel.: +358 9 1915 0339; fax: +358 9 1915 0330.
E-mail address: tommi.virtanen@helsinki.fi (T. Virtanen).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.04.017

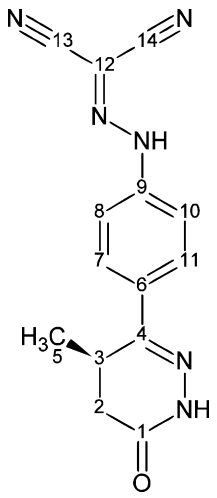

Fig. 1. Chemical structure of API-X and numbering of carbon sites.

cross-polarisation step required to transfer the magnetisation from abundant spins to rare spins, such as ^{13}C, may distort the relative intensities. Also, determining the total intensity of signal from a given site by integration may be difficult due to overlapping broad lines. A common strategy of treating the problem with quantitation in solid state NMR spectroscopy is using a known amount of some internal standard (*e.g.* magnesium stearate) added to the sample under study (Harris et al., 2005), or to calculate a correction based on cross-polarisation dynamics to the detected signal intensities (Gao, 1996). Deconvolution may be used for resolving overlapping lines, although care must be taken since differences may exist in the actual line shapes between sites. Spectra can often be simplified by utilising some spectral editing in applied pulse sequence, as for instance dipolar dephasing that leaves only signals from quaternary and methyl carbons to be observed.

In this work we used solid state ^{13}C CPMAS NMR spectroscopy to study the two polymorphs of an active pharmaceutical ingredient 2-[[4-[(4R)-4-methyl-6-oxo-4,5-dihydro-1H-pyridazin-3-yl]phenyl]hydrazinylidene]propanedinitrile (referred as API-X, CAS 141505-33-1, structure given in Fig. 1) designated as X1 and X2, from which, to our knowledge, solid state NMR data has not been previously published. Polymorphic mixtures of known weight composition were analysed with the aim being to produce quantitative information about the mixture. A chemometric method known as Direct Exponential Curve Resolution Algorithm (DECRA) has earlier been used to resolve spectrum from a mixture into its pure components (Zumbulyadis et al., 1999), and here its capability of yielding quantitative information is examined. In the case of this particular API methods for obtaining quantitative data are somewhat limited: DSC cannot be utilised for this purpose since no clear melting points for the polymorphs exist prior to their decomposition. There exists however a method based on XRPD that is able to yield high precision quantitative data (personal communication). There are, however, difficulties associated also to XRPD analysis in some occations (Padden et al., 1999). ^{13}C CPMAS NMR is therefore an interesting comparative method, and its suitability for study of this compound is to be tested here. As an addition to quantitation of the polymorphic mixtures a low-dose formulation of API-X was also examined in order to determine the possibility of API detection within the excipient matrix, and even identification of the polymorph present in formulation.

2. Materials and methods

The two pure polymorphic samples of API-X were provided by Orion Pharma. These samples were first characterised with XRPD by the provider (data not shown here), and mean particle sizes for X1 and X2 were determined (also by sample provider) with laser diffraction to be 55 μm and 30 μm. From the two pure polymorphs of API-X a series of mixtures was prepared, with X1 content varying from ~8% to ~80%. No grinding was applied during preparation to avoid conversion between polymorphic forms, only a careful stirring until the mixtures were homogeneous. The formulated product was provided in the form of tablets that were ground into a fine powder. In the formulated product, API content was less than 1% in a matrix consisting mainly of microcrystalline cellulose, other components were alginic acid and stearic acid. Approximately 200–300 mg of sample was used for every measurement.

2.1. Solid state NMR measurements

All spectra were measured with a Varian Unity Inova 300 spectrometer with a magnetic flux density of 7.05 T, using Varian's 7 mm VT/CPMAS double resonance probe. To ensure adequate magnetisation transfer to quaternary carbons we chose a relatively long cross-polarisation contact time of 4.5 ms for the pure polymorph samples and their mixtures. Acquisition time in all measurements was 50 ms, and spectral width was 50 kHz. A spinning rate of 5.5 kHz was used, to prevent the spinning sidebands present in the spectra from overlapping with the center band. Although spinning at a higher rate would improve the overall outlook of the spectrum by suppressing the sidebands, it could also induce conversions from one polymorphic form to another (Lee et al., 1999; Zell et al., 2000), and is therefore not preferable. All spectra were referenced to tetramethylsilane (TMS) via the spectrum of hexamethylbenzene by assigning 17.3 ppm for the methyl group signal. Better resolution, especially in the high field region, was achieved by measuring the spectra for pure polymorph samples using programmed decoupling (two-pulse phase modulation, TPPM). The number of scans accumulated for each spectrum was 2000, except for inversion recovery and arrayed contact time measurements for which only ~500 scans were collected in order to keep the measuring time within reasonable limits.

For both pure polymorphs the T_1 time constants for proton spin-lattice relaxation were determined to ensure usage of sufficiently long enough recycle delay between transients. Both X1 and X2 had relatively low $T_1(H)$ relaxation time constants, ~1.5 s and ~5 s. The necessary recycle delay is considered to be $\sim 5 \times T_1$. We, however, chose a delay of 10 s (a rather short time when considering X2) in order to obtain a reasonable overall acquisition time. For selected measurements a recycle delay of 25 s was also tested, and no significant difference in quantitation results were observed when compared to results obtained with a shorter delay.

2.2. Quantitation of the mixtures

Two different methods were utilised in quantitations: signal integration after protonated carbon suppression by dipolar dephasing with a correction to integrated intensities and a chemometric method DECRA (Windig and Antalek, 1997). In the former method a spectrum for the mixture is collected with a suitable delay between a cross-polarisation sequence and signal acquisition, allowing relaxation of those carbons (excluding methyl groups) with attached protons. The obtained spectrum therefore only includes only signals from quaternary and methyl carbons, resulting in fewer signal overlaps and allowing easier interpretation. For successful application in quantitation a specific site in a molecule that produces sufficiently separated signals for each polymorph that can be integrated or deconvoluted to yield relative amounts of polymorphic forms in the mixture must exist. DECRA, on the other hand, is capable of resolving a spectrum obtained from a mixture into a subspectra of its components, provided that some

experimental parameter exists that can be varied to yield a set of spectra with intensities exponentially dependent on that parameter. Such a parameter is for instance the delay time, τ, after the 180° pulse in a standard inversion recovery experiment for the measurement of the T_1-relaxation time constant, after subtraction of a reference spectrum (for which $\tau \gg T_1$). The polymorphs X1 and X2 show a significant difference in their T_1-relaxation rates, thus making τ a suitable parameter for DECRA analysis. DECRA has previously been applied to separate a spectrum of a mixture of polymorphs into subspectra (Zumbulyadis et al., 1999). In addition to determining the pure spectra of the components it is also capable of yielding the relative amounts of the components in the mixture. DECRA is rather insensitive to even a serious spectral overlap, and is therefore ideal for the separation of polymorphic mixtures, as long as the polymorphs have distinctive relaxation rates. DECRA calculation is not computatively and the implementation of the method is also easy. In this work calculations were carried using the Mathematica software (Wolfram Research, 2003).

Detected intensity in an experiment that uses cross-polarisation is affected by cross-polarisation dynamics, *i.e.* how efficient is the polarisation transfer from the abundant nuclei to in this case ^{13}C, and how fast is this polarisation leaked back to the environment by rotating frame relaxation. Therefore, should quantitative data be obtained it is essential to know the related time constants, T_{CH} and $T_{1\rho H}$, for these processes. For that purpose measurements with arrayed contact time (t_c) were performed, with t_c varying from 0.1 ms to 30 ms. Obtained time constants for cross-polarisation dynamics were then used to correct the integrated peak areas in quantitations as described by Gao (1996).

2.3. Correction to the mass fraction

Since dipolar dephasing was used in recording the spectra, it was also necessary to account for possible differences in the dephasing rates of the polymorphs. When the decoupling is turned off after contact time for a short time period (τ_{dd}), the signal is attenuated exponentially with a decay constant T_{dd}. The intensity after dipolar dephasing is therefore obtained as

$$I(\tau_{dd}) = I(t_c)\exp\left(-\frac{\tau_{dd}}{T_{dd}}\right), \quad (1)$$

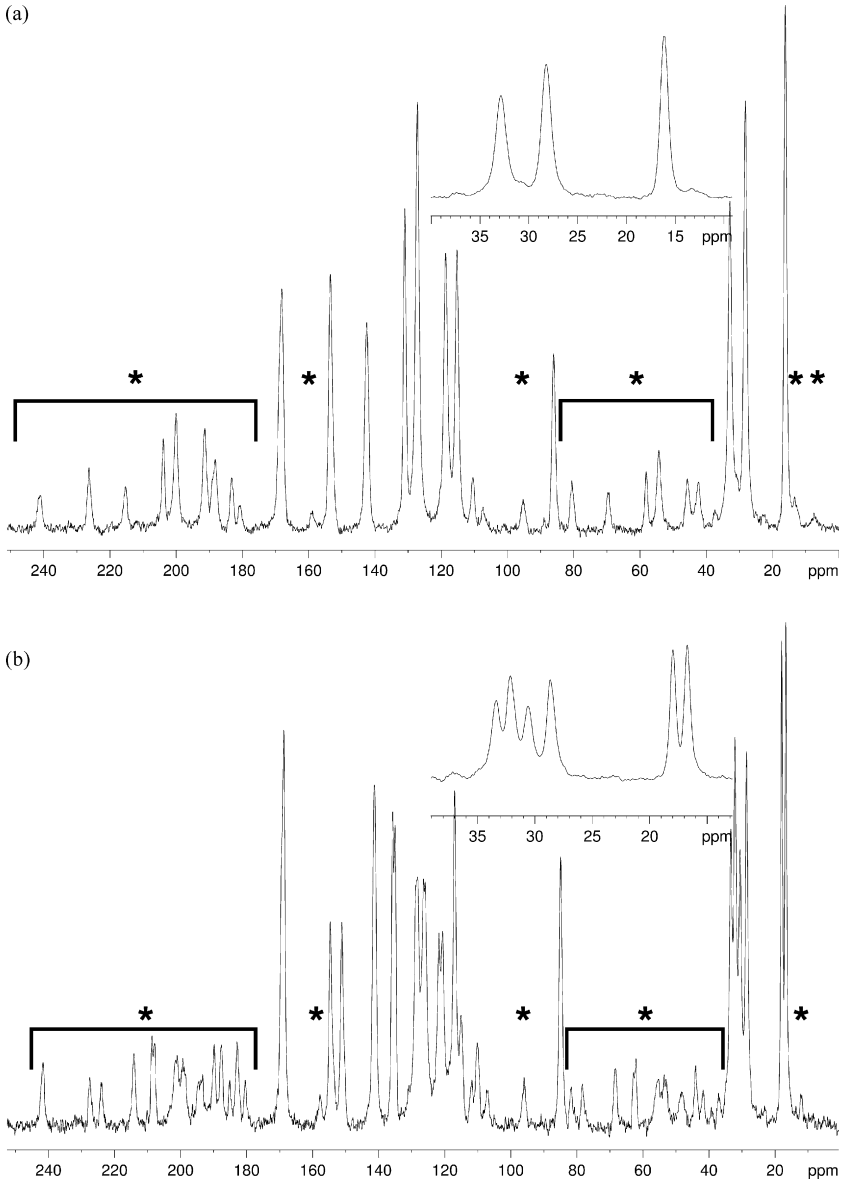

Fig. 2. Solid state NMR ^{13}C spectra of polymorphs X1 (a) and X2 (b). Expanded regions show the high field region where signal splitting due to differences in crystallographic arrangements can be observed. Signals marked with asterisk are spinning side bands.

where $I(t_c)$ is the intensity after the contact period of length t_c prior to dipolar dephasing. For two species A and B the ratio of their intensities after dephasing is then

$$\frac{I_A(\tau_{dd})}{I_B(\tau_{dd})} = \frac{I_A(t_c)}{I_B(t_c)} \exp\left(\frac{\tau_{dd}}{T_{B,dd}} - \frac{\tau_{dd}}{T_{A,dd}}\right) \quad (2)$$

When Eq. (2) is then combined with the result given by Gao, the mass fraction M_A/M_B of the species present in the mixture is obtained as

$$\frac{M_A}{M_B} = F_{A/B}\frac{I_A(t_c)}{I_B(t_c)} = F_{A/B}\frac{I_A(\tau_{dd})}{I_B(\tau_{dd})} \exp\left(\frac{\tau_{dd}}{T_{A,dd}} - \frac{\tau_{dd}}{T_{B,dd}}\right), \quad (3)$$

where $F_{A/B}$ is a shorthand notation for

$$F_{A/B} = \frac{\left(1 - \frac{T_{A,CH}}{T_{A,1\rho H}}\right)}{\left(1 - \frac{T_{B,CH}}{T_{B,1\rho H}}\right)} \frac{\left\{\exp\left(-\frac{t_c}{T_{B,1\rho H}}\right) - \exp\left(-\frac{t_c}{T_{B,CH}}\right)\right\}}{\left\{\exp\left(-\frac{t_c}{T_{A,1\rho H}}\right) - \exp\left(-\frac{t_c}{T_{A,CH}}\right)\right\}}, \quad (4)$$

and $T_{X,i}$ is the decay constant for process i and species X. Time constants for cross-polarisation dynamics were obtained by non-linear least squares fitting in the data obtained from variable contact time measurements. The time constant for the decay during dipolar dephasing was determined by variating the delay time τ_{dd} in the range of 10 μs, . . ., 100 μs, followed by a linear least squares fit after taking the logarithm of the measured intensities. Signal areas were used in calculations instead of peak heights, achieving better convergence in the non-linear least squares fitting.

3. Results

Solid state ^{13}C CPMAS NMR spectra for polymorphs X1 and X2 are shown in Fig. 2. These polymorphs have their own distinctive features in their spectrum, so they can be easily identified by solid state NMR methods. The (partial) assignments of the observed signals are given in Table 1, the numbering refers to that used in Fig. 1. Figs. 3 and 4 show the intensity as a function of contact time and logarithmic intensity as function of dipolar dephasing time. Time constants related to cross-polarisation dynamics and the rate of dipolar dephasing were calculated from the fitting parameters, and are given in Table 2.

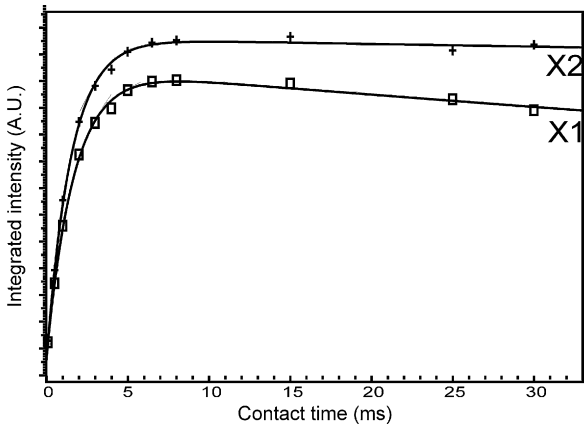

Fig. 3. Integrated signal intensities from carbon site C6 as a function of contact time. While both polymorphs have comparable cross-polarisation rates, X1 has clearly a faster rotating frame relaxation. Time constants related to cross-polarisation dynamics were obtained from a non-linear least squares analysis.

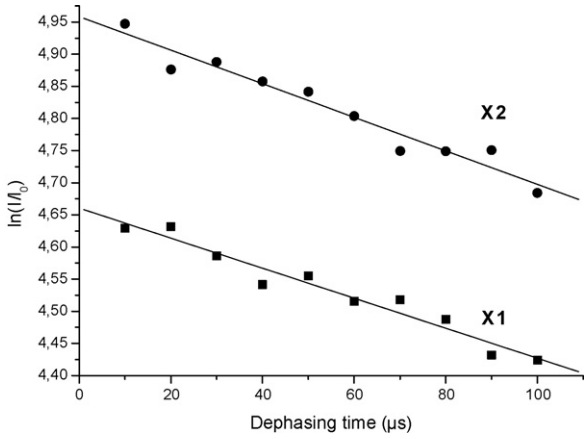

Fig. 4. Logarithmic intensity from the C6 carbon site as a function of dipolar dephasing delay time for polymorphs X1 and X2. Exponential time constant for decay was obtained from this data using linear least squares line fitting.

When dipolar dephasing was applied, distinctive signals from aromatic carbon C6 can be seen as a singlet at 131.0 ppm for X1 and as a doublet at (135.8, 135.1) ppm for X2. These were sufficiently separated to be integrated and these signals, including their spinning side bands, were selected for use in quantitations. For DECRA analysis we chose a low field region of approximately 100–175 ppm. As a result of the analysis the pure spectra of the components are resolved, and their relative contribution to the observed intensity in the spectrum at each time point is also obtained. When a linear least squares fitting is performed on the logarithm of this concentration profile, the composition of the mixture can be estimated from the intercepts of the concentration axis.

Quantitation results for the polymorphic mixtures are presented in Table 3, with the DECRA results analysis shown in Fig. 5. Fig. 6 presents the accuracy of the used quantitation methods. Finally, the

Table 1
Assignments of the solid state NMR spectra of API-X polymorphs. For numbering, see Fig. 1. With data collected for present work, assignments cannot, however, be made completely ambiguous (see text for details).

X1		X2	
ppm	Carbon No	ppm	Carbon No
168.05	1	168.78	1
153.45	4	154.70	4
142.44	9	151.22	4
130.99	6	141.39	9
127.23	7, 11	135.81	6
118.65	8, 10	135.12	6
118.54	13, 14	128.28	7, 11
116.11	13, 14	126.46	7, 11
115.26	8, 10	121.81	8, 10
110.40	13, 14	120.72	8, 10
107.24	13, 14	117.04	8, 10
86.05	12	115.09	13, 14
32.93	2	111.94	13, 14
28.27	3	110.16	13, 14
16.18	5	107.29	13, 14
		84.95	12
		33.41	2, 3
		32.16	2, 3
		30.62	2, 3
		28.68	2, 3
		18.00	5
		16.74	5

Table 2
Time constants for cross-polarisation dynamics and for dipolar dephasing.

	T_{cp} (ms)	$T_{1\rho}$ (s)	T_{dd} (ms)
X1	1.05 (0.15)	0.49[a]	0.43 (0.07)
X2	1.08 (0.11)	1.51[a]	0.38 (0.06)

[a] Error for $T_{1\rho}$ not available due to limitations in the applied fitting model.

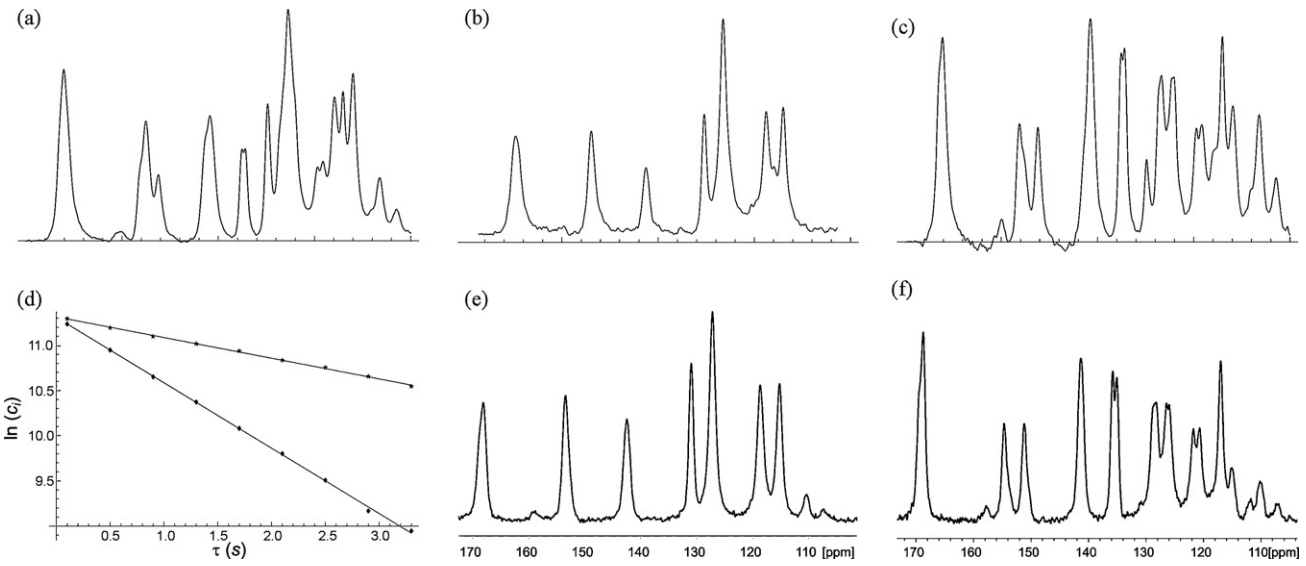

Fig. 5. Output from DECRA. Low field region from a spectrum of a mixture with 48% X1 by weight was given as input (a). Resolved components: X1 (b) and X2 (c). Corresponding measured true pure low field regions for X1 and X2 are presented in (e) and (f). In (d) the concentration profiles are shown, with c_i being the contribution of the resolved pure spectrum of species i to the total signal. The actual composition of the mixture is calculated as an extrapolation to zero time.

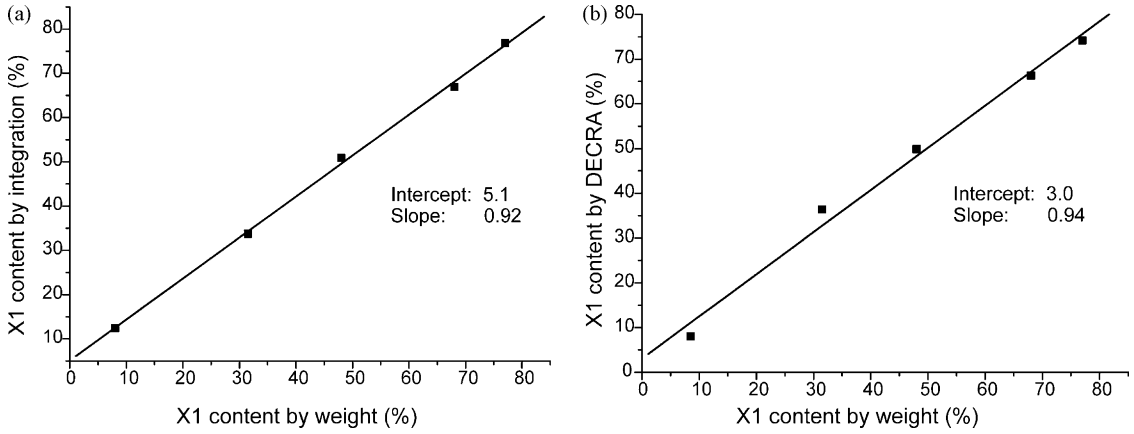

Fig. 6. Comparison of the quantitation results. In the figures the weighted mass fraction of X1 is plotted against integration result (a) and DECRA result (b).

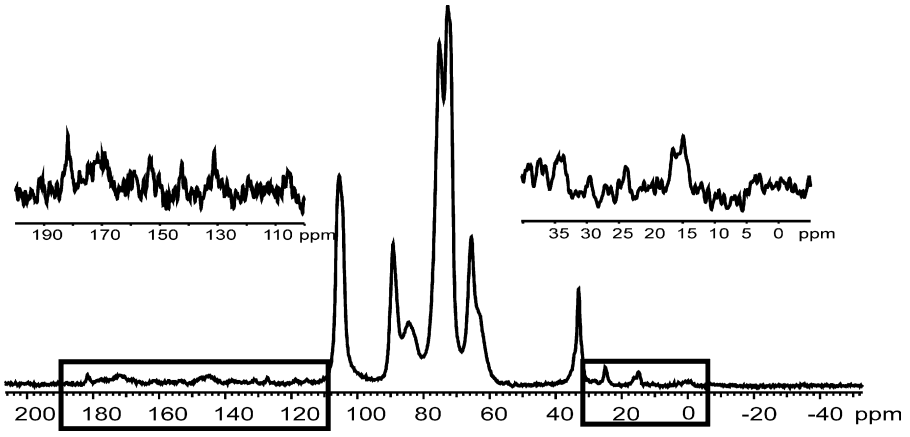

Fig. 7. Solid state NMR spectrum of API-X in formulation. Besides the dominating signals from the drug matrix traces of API can be seen in both low and high field regions. Expanded views show signals from the boxed regions obtained with dipolar dephasing.

Table 3
Quantitation results for polymorphic mixtures as obtained by signal integration and by DECRA. Error limits for X1 content by DECRA calculation are obtained from line fitting to concentration profiles (see Fig. 4d). Error in X1 content obtained by integration was estimated to be within 2%.

X1 content by weight (%)	Integration	DECRA
77.0	76.8	74.2(0.3)
68.0	66.9	66.3(0.5)
48.0	50.9	49.9(0.5)
31.5	33.7	36.4(0.4)
8.5	N/A[a]	8.05(0.07)
8.0	12.5	N/A[a]

[a] Values missing due to spectrometer instability during measurement.

spectrum obtained from the formulated product is shown in Fig. 7. Expanded views from the low and high field regions included in the figure are obtained with dipolar dephasing.

4. Discussion

4.1. Characteristics of the spectra

The most notable difference between the spectra obtained from X1 and X2 is the splitting of the signals into doublets in the X2 spectrum, indicating that two molecules are present in the asymmetric unit of X2. The splitting effect can be seen on the signals in the aromatic region, and also in the signals from the $-CH_2-$ and the methyl groups at 15–35 ppm when programmed decoupling was used. Also, significant changes in chemical shift values occur between the two polymorphs, especially with carbon C6 for which a difference over 4 ppm appears in high field region.

Magic angle spinning does not completely average out the dipolar coupling between ^{14}N and ^{13}C. This can be seen as splitting or broadening of signals from those sites that have ^{14}N attached directly to them, as often reported in the literature (Opella et al., 1979; Reutzel-Edens et al., 2003; Hughes et al., 2002; Mooibroek and Wasylishen, 1987). The presence of this residual coupling can be seen in the case of API-X in the signals C13 and C14, both giving the typical 2:1 doublet for this kind of interaction. In other carbon sites directly bonded to nitrogen this splitting effect is not observed, probably due to different relative orientations of the dipolar coupling tensor and quadrupolar interaction tensor at these sites. In the case of X1 only one of these doublets can be observed in the regular spectrum, signal from the aromatic carbons C8 and C10 obscure the other. It is only when dipolar dephasing is applied that the second doublet from the pair C13/C14 also becomes visible (Fig. 8).

Suggested assignments of the solid state NMR signals are given in Table 1. These are based on liquid state NMR assignments (data not shown), dipolar dephased spectra, and effects observed from residual couplings. It should be recognised that assignment of a solid state spectrum is not generally a trivial task. Since, for instance, differences in hydrogen bonding patterns between polymorphs may have a large effect on observed chemical shift values, even to the extent that signal cross-over for two sites may occur, it is preferable to also have some computational data supporting the assignment task. Since we did not gather such data, assignments presented here can only be considered qualitative.

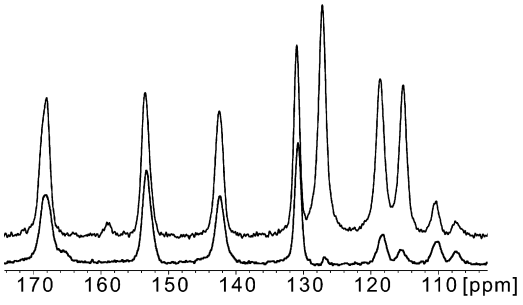

Fig. 8. Low field region of X1. Dipolar dephased spectrum (on bottom) and normal spectrum (on top). Both carbons 13 and 14 give an asymmetric doublet to the spectrum due to residual coupling to ^{14}N, as can be seen in the dipolar dephased spectrum.

4.2. Quantitation

Fig. 6 demonstrates that both quantitation methods used show good accuracy with the weighted compositions of the samples. The root mean square (RMS) value calculated from the results obtained with DECRA was slightly higher (6.21) compared to corresponding value calculated from signal integration results (5.89). The largest single deviation from the weighted amount, on the other hand, comes from integration of the mixture with 8.5% X1, giving an over-estimated result of 12.5%. In the case where X2 was the minor component the result from integration was accurate. DECRA, on the other hand, also gave a fairly accurate result for the low X1 case. It appears that the correction calculated for the mass fraction using Eq. (3) only has a minor effect on the outcome of the quantitation, only a 1.3% average difference in the relative amounts. This is probably due to the similarity of the cross-polarisation behaviour of the polymorphs in this case.

Accuracy of the integration process was tested with a 31.5% X1 sample by carrying out the processing of the spectrum from the raw data to the final integration step (including drift correction, base line correction, phasing of the spectrum and small variations in integral regions) with several repetiotions. For seven repetitions, standard deviation of 1.8% from the average integral value was obtained. Similar analysis for results obtained with DECRA yields a standard deviation of 1.0%. This indicates that DECRA is somewhat less susceptible to small changes in processing conditions than the direct integration of the signals. However, since the measuring time for spectral data required by DECRA is much longer (from 1 day to even 4 days, depending on the consentration of the minor component) compared to that of collecting a basic dipolar dephased spectrum (20 h), practicality of DECRA is limited.

When resolved subspectra obtained from DECRA are compared to true pure spectra it is apparent that DECRA succeeds very well in the separation of spectra. Separation is not accurate, however, in all areas of the spectra, for instance the signal originating from carbons C13/C14 is transferred completely to the X2 component. The use of DECRA therefore requires some amount of calibration with samples of known composition, since not every spectral region was suitable for quantitation purposes. For instance, when the whole spectral range was used in the calculation of relative contributions of the components, the deviation from the weighted mass fraction was in some cases more than 10%. From the high field region (~10 ppm to 40 ppm) it was possible to select a range that yielded better accuracy than these results presented, however, this region suffered from poor repeatability. By making a small change in the selected range a notable change emerged in quantitation result (~5%) The selected region 100–175 ppm, gave good reproducibility with reasonable accuracy in the results.

DECRA requires an initial guess for the number of components present in the mixture. This is no problem even when the actual number is unknown since the calculation is very fast. It is easy to use the trial and error method, and in every case the best possible choice is not necessarily the actual true number of components. We used three components, since this gave the best overall outcome when all compositions were considered.

Despite the shortcomings mentioned above, DECRA is a worthwhile option for quantitative work, since in general suitable clearly resolved signals may not be present in spectrum that can be inte-

grated, or even deconvoluted, reliably. As a self-modeling method DECRA also circumvents some of the problems associated with quantitation by integration, since it does not rely on relative intensities of the components in a mixture spectrum. Although the calculated correction to the mass fraction based on cross-polarisation dynamics had no significant effect on the outcome, it does not lower the importance of accounting for these matters when using signal integration for quantitation. This requires careful calibration of the system and determination of the parameters relevant to the cross-polarisation experiment. Also, the inclusion of the spinning sidebands into the integral values is important, and depending on the amount of chemical shift anisotropy of the site in question and on complexity of the spectrum this may become a serious problem. What also makes DECRA attractive is the uniqueness of the solution it gives for a given spectral range, this cannot be expected from results obtained using deconvolution with an even slightly complicated set of overlapping signals.

4.3. Formulated product

In the spectrum of a formulated API-X (~0.7% by weight) the main contribution to the overall signal at ~60 ppm to 110 ppm comes from microcrystalline cellulose used as a matrix (see Fig. 7). Another clearly observed signal comes from magnesium stearate at ~37 ppm. Since none of the excipients have resonances in the low field region, the signal observed between ~130 ppm and 155 ppm can be identified as originating from the API. Another contribution from API can be found at the high field region close to 15 ppm. Observed signals for API are, however, too weak to even be able to give exact values for peak locations. From a dipolar dephased spectrum the low field signals can be more readily seen, but in every case the signal to noise ratio (S/N) is too low (7.9) to make any conclusions about which polymorph is present in the sample. The number of transients for this experiment was 30,000 (measuring time 24 h), it is thus not feasible to try to improve the S/N ratio by simply increasing the number of scans. Despite the low S/N, however, the presence of the API-X in this formulation can be detected with solid state NMR methods, even with such a low content as 0.7% by weight.

5. Conclusions

Two polymorphs, X1 and X2, of an active pharmaceutical ingredient API-X were studied using solid state ^{13}C CPMAS NMR spectroscopy. The spectral features of the two polymorphs were very different, so the identification based on their ^{13}C CPMAS NMR spectra can be easily done. Samples with varying polymorph composition were analysed quantitatively, and a formulated API was also studied. While the quantitation results using integration for low X1 content sample were not satisfactory, the overall quantitative results showed reasonable accuracy. In the formulated sample it was relatively easy to detect signals of the API, since none of the excipient signals were close to overlapping them. Although the signal to noise ratio was not adequate enough for reliable identification of the polymorph present in the formulation, it was demonstrated that it is possible to observe the presence of the API in this formulation.

The data obtained with solid state ^{13}C CPMAS NMR is a valuable addition to any information obtained with other spectroscopical tools. The ability to distinguish the polymorphic components by simple inspection of the spectra, and the capability for yielding quantitative information about polymorphic mixtures are aspects that bring great practical importance to solid state NMR in the study of pharmaceutical compounds.

Acknowledgments

Finnish Funding Agency for Technology and Innovation (TEKES) is greatly acknowledged for financing this study. Orion Pharma and Fermion are acknowledged for providing the API materials and liquid state NMR spectra related to them.

References

Agatonovic-Kustrin, S., Rades, T., Wu, V., Saville, D., Tucker, I., 2001. Determination of polymorphic forms of ranitide-HCl by DRIFTS and XRPD. J. Pharm. Biomed. Anal. 25, 741–750.
Brittain, H.G., Bogdanowich, S.J., Bugay, D.E., DeVincentis, J., Lewen, G., Newman, A.W., 1991. Physical characterization of pharmaceutical solids. Pharm. Res. 8, 963–973.
Bugay, D.E., 2001. Characterization of the solid-state: spectroscopic techniques. Adv. Drug Deliv. Rev. 48, 43–65.
Gamberini, M., Baraldi, C., Tinti, A., Rustichelli, C., Ferioli, V., Gamberini, G., 2006. Solid state characterizaton of chloramphenicol palmitate. Raman spectroscopy applied to pharmaceutical polymorphs. J. Mol. Struct. 785, 216–224.
Gao, P., 1996. Determination of the composition of delavirdine mesylate polymorph and pseudopolymorph mixtures using ^{13}C CP/MAS NMR. Pharm. Res. 13, 1095–1104.
Gervais, C., Profeta, M., Lafond, V., Bonhomme, C., Azaïs, T., Mutin, H., Pickard, C.J., Mauri, F., Babonneau, F., 2004. Combined ab initio computational and experimental multinuclear solid-state magnetic resonance study of phenylphosphonic acid. Magn. Reson. Chem. 42, 445–452.
Gustafsson, C., Lennholm, H., Iversen, T., Nyström, C., 1998. Comparison of solid-state NMR and isothermal microcalorimetry in the assessment of the amorphous component of lactose. Int. J. Pharm. 174, 243–252.
Harris, R.K., 1985. Quantitative aspects of high-resolution solid-state nuclear magnetic resonance spectroscopy. Analyst 110, 649–655.
Harris, R.K., 2006. NMR studies of organic polymorphs & solvates. Analyst 131, 351–373.
Harris, R.K., Hodgkinson, P., Larsson, T., Muruganantham, A., 2005. Quantification of bambuterol hydrochloride in a formulated product using solid-state NMR. J. Pharm. Biomed. Anal. 38, 858–864.
Hughes, C., Pratima, R., Karlsson, T., Levitt, M., 2002. Double-quantum solid-state NMR of ^{13}C spin pairs coupled to ^{14}N. J. Magn. Reson. 159, 25–35.
Kordikowski, A., Shekunov, T., York, P., 2001. Polymorph control of sulfathiazole in supercritical CO_2. Pharm. Res. 18, 682–688.
Lee, H.-H., Kimura, K., Takai, T., Senda, H., Kuwae, A., Hanai, K., 1999. Polymorphism of phenylpyruvic acid studied by IR, Raman and solid state ^{13}C NMR spectroscopy. Spectrochim. Acta Part A 55, 2877–2882.
McMahon, L.E., Timmins, P., Williams, A.C., York, P., 1996. Characterization of dihydrates prepared from carbamazepine polymorphs. J. Pharm. Sci. 85, 1064–1069.
Mooibroek, S., Wasylishen, R.E., 1987. A carbon-13 nuclear magnetic resonance study of solid tetracyclines. Can. J. Chem. 65, 357–362.
Offerdahl, T.J., 2006. Solid-state nuclear magnetic resonance spectroscopy for analyzing polymorphic drug forms and formulations. Pharm. Technol., 24–42.
Opella, S., Frey, M., Cross, T., 1979. Detection of individual carbon resonances in solid proteins. J. Am. Chem. Soc. 101, 5856–5857.
Padden, B.E., Zell, M.T., Dong, Z., Schroeder, S.A., Grant, D.J.W., Munson, E.J., 1999. Comparison of solid-state ^{13}C NMR spectroscopy and powder X-ray diffraction for analyzing mixtures of polymorphs of neotame. Anal. Chem. 71, 3325–3331.
Patel, A.D., Luner, P.E., Kemper, M.S., 2000. Quantitative analysis of polymoprhs in binary and multi-component powder mixtures by near-infrared reflectance spectroscopy. Int. J. Pharm. 206, 63–74.
Raghavan, K., Dwivedi, A., Campbell Jr., G.C., Johnston, E., Levorse, D., McCauley, J., Hussain, M., 1993. A spectroscopic investigation of losartan polymorphs. Pharm. Res. 10, 900–904.
Reutzel-Edens, S.M., Kleemann, R.L., Lewellen, P.L., Borghese, A.L., Antoine, L.J., 2003. Crystal forms of LY334370 HCl: isolation, solid-state characterization, and physicochemical properties. J. Pharm. Sci. 92, 1196–1205.
Roberts, S.N.C., Williams, A.C., Grimsey, I.M., Booth, S.W., 2002. Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry—exploring preferred orientation effects. J. Pharm. Biomed. Anal. 28, 1149–1159.
Sheikhzadeh, M., Rohani, S., Jutan, A., Manifar, T., Murthy, K., Horne, S., 2006. Solid-state characterization of buspirone hydrochloride polymorphs. Pharm. Res. 23, 1043–1050.
Smith, J.R., Xu, W., Raftery, D., 2006. Analysis of conformational polymorphism in pharmaceutical solids using solid-state NMR and electronic structure calculations. J. Phys. Chem. B 110, 7766–7776.
Stephenson, G.A., Forbes, R.A., Reutzel-Edens, S.M., 2001. Characterization of the solid state: quantitative issues. Adv. Drug Deliv. Rev. 48, 67–90.
Tozuka, Y., Ito, A., Seki, H., Oguchi, T., Yamamoto, K., 2002. Characterization and quantitation of clarithromycin polymorphs by powder X-ray diffractometry and solid-state NMR spectroscopy. Chem. Pharm. Bull. 50, 1128–1130.
Windig, W., Antalek, B., 1997. Direct exponential curve resolution algorithm (DECRA): a novel application of the generalized rank annihilation method for

a single spectral mixture data set with exponentially decaying contribution profiles. Chemom. Intell. Lab. Syst. 37, 241–254.
Wolfram Research, Inc., 2003. Mathematica, version 5.0. Wolfram Research, Inc., Champaign, Illinois.
Yates, J.R., Dobbins, S.E., Pickard, C.J., Mauri, F., Ghi, P.Y., Harris, R.K., 2005. A combined first principles computational and solid-state NMR study of a molecular crystal: flurbiprofen. Phys. Chem. Chem. Phys. 7, 1402–1407.
Zell, M.T., Padden, B.E., Grant, D.J.W., Schroeder, S.A., Wachholder, K.L., Prakash, I., Munson, E.J., 2000. Investigation of polymorphism in aspartame and neotame using solid-state NMR spectroscopy. Tetrahedron 56, 6603–6616.
Zumbulyadis, N., Antalek, B., Windig, W., Scaringe, R.P., Lanzafame, A.M., Blanton, T., Helber, M., 1999. Elucidation of polymorph mixtures using solid-state ^{13}C CP/MAS NMR spectroscopy and direct exponential curve resolution algorithm. J. Am. Chem. Soc. 121, 11554–11557.