# Quantitative Nuclear Magnetic Resonance Analysis of Solid Formoterol Fumarate and Its Dihydrate

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*Received 31 January 2003; revised 16 June 2003; accepted 17 June 2003*

**ABSTRACT:** Carbon-13 cross-polarization magic-angle spinning nuclear magnetic resonance spectra of anhydrous formoterol fumarate and the dihydrate are presented, together with some relaxation time measurements. The latter enabled quantitation of mixtures of the anhydrate and dihydrate to be made. Quantitative nuclear magnetic resonance measurements were then performed on mixtures of the two forms formulated in lactose. Relative amounts of the forms could be assessed at a total formulation level of 2%, whereas the dihydrate on its own in lactose was detectable at the 0.45% level. The optimum experiment involves dipolar dephasing, because that minimizes the intensity of signals from the lactose. © 2003 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 92:2487–2494, 2003

**Keywords:** analytical chemistry; NMR spectroscopy; solid-state NMR; relaxation time; polymorphism; hydrates; hydration; formulation; excipients

## INTRODUCTION

Quantitation of drug molecules in formulated products is important in pharmaceutical chemistry, as is the detection and characterization of polymorphic forms (including pseudo-polymorphs, i.e., solvates). Such measurements are of special significance for polymorphic transformations or hydration/dehydration in formulations because these relate to drug efficacy and regulatory compliance.[1] There are many methods of approaching such problems.[2,3] Powder X-ray diffraction (XRD) is commonly used but suffers from a number of disadvantages because relative quantitation is not straightforward and is especially problematic if amorphous or disordered forms are involved. However, in recent years, magic-angle spinning (MAS) NMR has been shown[4] to be a powerful tool for investigating such problems. It is highly discriminatory, can be used in the presence of excipients, responds well to amorphous components, and can be made quantitative. However, it is not, in principle, a highly receptive method and the limits of detection in formulated products have in most cases not been established. Moreover, quantitation requires a knowledge of relaxation times. If cross polarization (CP) is used, the CP rates for different signals also must be known, at least qualitatively.

We have used $^{1}H \rightarrow {}^{13}C$ CPMAS NMR to establish the conditions for and limits of quantitation of a drug substance incorporated into a common excipient, namely α-lactose monohydrate, using formoterol fumarate, **I**, as an example. The case also exemplifies the use of solid-state NMR for the study of hydration of a drug substance. The relevant forms of formoterol fumarate are the anhydrous compound, polymorph I (identified as FFA here), and the dihydrate (designated FFD). FFA is thermodynamically stable under dry conditions. Under humid conditions, FFD is the

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Journal of Pharmaceutical Sciences, Vol. 92, 2487–2494 (2003)

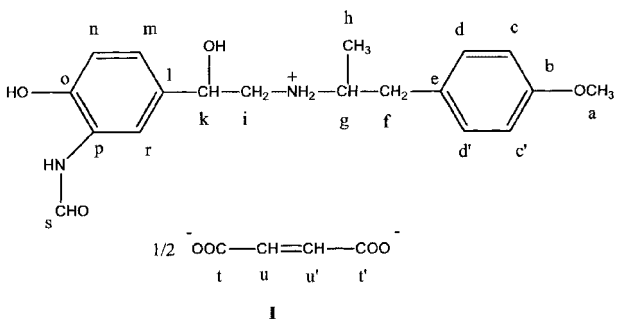
thermodynamically stable form. Formoterol fumarate has the CAS number 43229-80-7 and the full name for the dihydrate is: (R*,R*)-(±)-*N*-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide, (*E*)-2-butendioate (2:1), dihydrate.

Formoterol is a rapid-acting and long-lasting $\beta_2$-agonist used in asthma therapy because of its bronchodilating effect.[5] In comparison with other $\beta_2$-agonists, formoterol has a unique pharmacological profile, as rapid-acting as the short-acting $\beta_2$-agonist, salbutamol, and with at least 12 h of bronchodilator effect.[6] This unique profile may allow formoterol to be used not only as a maintenance treatment but also as a reliever.

## EXPERIMENTAL

### Materials

FFD was supplied by AstraZeneca Bulk Production Sweden. FFA was produced by placing FFD at 80°C in a flow of nitrogen to drive off the water, followed by suspension in water-free ethyl acetate for 24 h. The two pure forms are characterized by their powder X-ray diffractograms (Fig. 1). A sample containing a mix of FFD and FFA was produced by withdrawing the FFD sample from storage at 80°C in a nitrogen flow before all water was driven off, followed by suspension in ethyl acetate for 24 h. A sample containing 0.45% mass ratio of FFD in α-lactose monohydrate was collected direct from an AstraZeneca Oxis Turbuhaler® production batch, whereas a sample containing 2% mass ratio of the FFD/FFA mix in α-lactose monohydrate was prepared by manually dry-mixing the components.




I

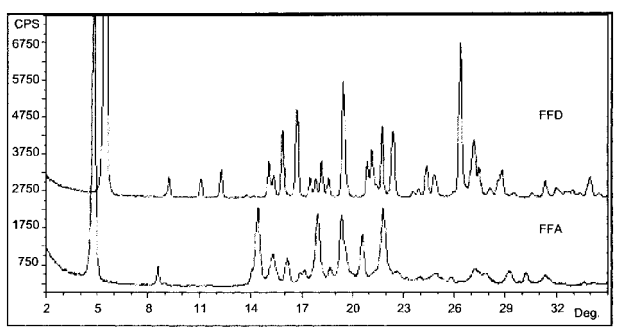



**Figure 1.** X-ray powder diffractograms for formoterol fumarate anhydrate (FFA) and formoterol fumarate dihydrate (FFD).

### Methods

#### *Powder XRD*

The diffractograms were obtained with a Scintag XDS 2000 θ-θ diffractometer (Scintag Inc., USA) equipped with a liquid-nitrogen-cooled solid-state germanium detector using CuKα radiation (1.5418 Å, 45 kV, and 30 mA) and spinning sample holders. The studied angular range was 2–35° (2θ). A continuous scan rate of 1°/min and a step size of 0.03° were used. On the primary side, 2- and 4-mm slits were used, with 0.5- and 0.3-mm slits on the secondary side.

#### *Solution-State NMR*

The proton-decoupled $^{13}C$ NMR spectrum was obtained using a 400-MHz Bruker Avance DRX NMR spectrometer, equipped with a 5-mm $^{13}C$, $^{19}F$, $^{31}P/^{1}H$ QNP probehead. The operating frequency for $^{13}C$ is 100.6 MHz. The repetition time was 4.3 s and 2 k transients were accumulated. The sample (of FFD) was dissolved in $CD_3OD$, and for reporting the chemical shifts on the δ scale, the $CD_3$ solvent resonance was set to 49.0 ppm. The operating probe temperature was 25°C. The number of $t_1$ points used for the HMQC and HMBC experiments[7,8] was 256 in each case.

#### *Solid-State NMR*

Proton-decoupled $^{13}C$ CPMAS spectra at 75.43 MHz of the solid samples were obtained using a Varian UnityPlus 300 spectrometer with 7-mm o.d. zirconia rotors. Rotor end-caps were made of kel-F, and spin rates were in the range 4–5 kHz. Contact times were 1 ms and recycle delays were 3 s (2 s for the low-temperature spectrum). The number of transients accumulated ranged from 200 to 20,000. Decoupling and Hartmann-Hahn matched[9] r.f. powers corresponded to ca. 60 kHz. The flip-back sequence[10] was used and, in some cases, TOSS[11] (total suppression of spinning sidebands). The dipolar dephasing sequence[12] was

used to suppress signals from CH and $CH_2$ carbons. The normal operating probe temperature was ca. 22°C. The spectrum at ca. −50°C was obtained by cooling the nitrogen gas used for spinning the sample. Chemical shifts are reported with respect to the signal for $(CH_3)_4Si$ but were measured via the CPMAS spectrum of a replacement sample of powdered adamantane, with its high-frequency signal taken to be at $\delta = 38.4$ ppm.

## RESULTS AND DISCUSSION

The solution-state $^{13}C$ spectrum of formoterol fumarate hydrate in $CD_3OD$ is displayed in Figure 2. Separate signals are seen for most carbons, but double-intensity resonances are observed for the pairs of phenylene ring carbons C-c,c′ and C-d,d′. [For atom lettering, see **I**.] The fumarate ion gives rise to only two (double-intensity) signals, as expected from symmetry considerations. Assignment of the signals was derived from the coupled proton spectrum via HMQC and HMBC experiments. The chemical shifts are listed in Table 1.

Figure 3a shows the $^{13}C$-{$^1H$} CPMAS spectrum of FFD, whereas Figure 3b,c show subspectra for the nonprotonated and protonated carbons, respectively (obtained using the dipolar dephasing experiment and subtraction procedures, respectively). As expected, methyl carbon signals appear in both Figures 3b and 3c, although more intensely in the former than in the latter. The purposes of the dipolar dephasing experiment are: (a) to assist in spectral assignment, and (b) to provide the basis of the optimum quantitation experiment for the drug in admixture with lactose (as will be discussed below). The chemical shifts and assignments are presented in Table 1. Peak assignment is assisted by the expectation that signals from carbons directly bonded to nitrogen (of which there are four) are broadened by residual dipolar coupling[13] to quadrupolar $^{14}N$, which cannot be completely eliminated by MAS. Apart from the effect of coupling to nitrogen, in general each formoterol carbon gives rise to a single signal, so it can be concluded that the crystallographic asymmetric unit contains one whole molecule-ion. However, a single resonance is obtained from the two carboxyl carbons of the fumarate ion, as is also true for the two protonated fumarate carbons, so it may be deduced that the asymmetric unit contains only half a fumarate ion. It appears that C-g and C-i give overlapping signals at $\delta_C = 57.0$ ppm. No peaks can be detected in Figure 3a for the four protonated carbons of the phenylene ring, and it is deduced that at ambient probe temperature there is rapid internal rotation of this ring about the C-b to C-e axis and/or of the methoxy group.[14–16] This suggestion is proved by a spectrum obtained at ca. −50°C, shown in Figure 3d, which reveals four more aromatic peaks. Table 1 also compares the chemical shifts for the solution and solid states. The shifts to high frequency (by 2.1 ppm) for the fumarate carboxyl C-t,t′ and to low frequency (by 2.9 ppm) for the formoterol C-k presumably arise from the hydrogen bonding in the solid state.[17] The substantial effects of phase change on the shifts for C-b, C-f, and C-i can probably be attributed to variations in conformations. The assignment of our solid-state spectra assumes that the signals for C-m, C-n, and C-r occur in the same order as for the solution, but the change of phase appears to cause substantial shifts for these resonances (as well as for the average of each of the c, c′ and d, d′ pairs of signals), the origin of which is obscure. The lack of any significant effect of crystallization on the chemical shift of the aldehyde carbon is surprising. It suggests that there is strong hydrogen bonding involving this carbon in the solution state as well as the solid. However, it should be noted that there may be systematic differences in the shifts for the two phases arising from the different referencing methods.

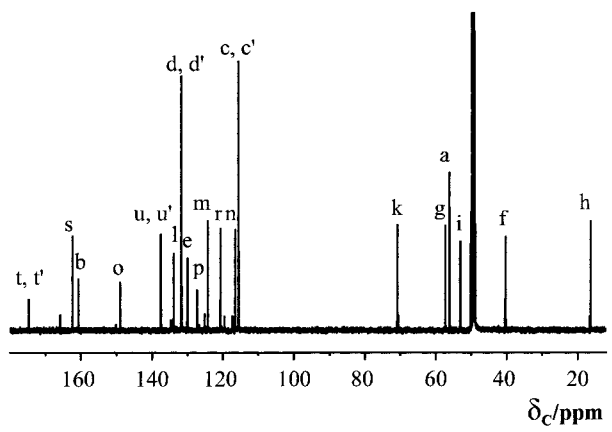



**Figure 2.** Proton-decoupled $^{13}C$ spectrum of a solution of formoterol fumarate. The quaternary carbons have lowered intensity, presumably because the recycle delay is not adequate for full relaxation.

Figure 4 is the CPMAS spectrum for 0.45% (by mass) of FFD in lactose, with no attempt at suppression of the lactose signal, no TOSS, and no dipolar dephasing. The lactose signals dominate the spectrum and are off-scale on this plot. The

**Table 1.** Proton and Carbon Chemical Shifts for FFA in Solution and ($^{13}$C Only) in the Solid State

| Atom Letter[a] | Solution | | $^{13}$C Shift/ppm for FFD (Solid) | Difference/ppm | $^{13}$C Shift/ppm for FFA (Solid) |
|---|---|---|---|---|---|
| | $^{1}$H Shift/ppm | $^{13}$C Shift/ppm | | | |
| a | 3.78 | 56.1 | 55.2 | +0.9 | 55.2 |
| b | — | 160.7 | 158.5 | +2.2 | 159.2 |
| c | 6.89 | 115.6 | 107.2[b] | +8.4 | ~114 |
| c′ | 6.89 | 115.6 | 118.6[b] | −3.0 | ~114 |
| d | 7.17 | 131.8 | 127.5[b] | +4.3 | ~127 |
| d′ | 7.17 | 131.8 | 130.9[b] | +0.9 | ~127 |
| e | — | 130.0 | 129.2 | +0.8 | ~129 |
| f | 2.70 and 3.11 | 40.3 | 35.5 | +4.8 | 40.0 and 36.1 |
| g | 3.50 | 57.3 | 57.0 | +0.3 | 53.6[c] or 49.9[c] |
| h | 1.22 | 16.4 | 16.2 | +0.2 | 17.2 and 14.5 |
| i | 3.18 | 53.1 | 57.0 | −3.9 | 53.6[c] or 49.9[c] |
| k | 4.80 | 70.8 | 67.9 | +2.9 | 72.6 and 68.3 |
| l | — | 133.9 | 132.7 | +1.2 | 132.3 |
| m | 7.06 | 124.3 | 121.2[c] | +3.1 | 122.9 |
| n | 6.89 | 116.6 | 112.2[c] | +4.4 | ~114 |
| o | — | 148.9 | 147.6 | +1.3 | 147.1 |
| p | — | 127.3 | 126 | ~1 | ~127 |
| r | 8.14 | 120.7 | 116.4[c] | +4.3 | 120.8 |
| s | 8.33 | 162.4 | 163 | ~0 | ~159 |
| t,t′ | — | 174.7 | 176.8 | −2.1 | 175.1 |
| u,u′ | 6.73 | 137.5 | 138.3 | −0.8 | 137.7 |

[a]For carbon atom letters, see **I**. The bonded hydrogen atoms are given the same letter.
[b]It is not known how the c and c′ resonances pair with those of d and d′ for atoms ortho to one another.
[c]Assignments not certain.

lactose produces some low-intensity spinning sidebands and the positions of these are marked with asterisks. In addition to the lactose-related signals, there are signals that can be assigned to FFD (marked with vertical arrows in Fig. 4). This spectrum took 15 h to obtain but there is no doubt that the FFD can be detected. Of course, that is not to say that it could be accurately quantified at that level. In this case, the sample (reportedly) contains only the dihydrate. If a mixture of dihydrate/anhydrate were present this would effectively "dilute" the signal (the anhydrate signals are also broader; see below) and would make detection of the drug more difficult. Certainly, minor components (up to, say, 25% of the drug molecule) would not be detectable, and anything approaching a 1:1 mixture might divide the signal enough to make the identity of the components uncertain. As will be shown below, dipolar dephasing can be used to discriminate against lactose signals, but this experiment is unlikely to lead to detectability at levels significantly lower than 0.45% because there is inevitably some loss of intensity for the FFD quaternary carbons during the dephasing delay. The ability of $^{13}$C NMR to quantify mixtures of polymorphs is clearly comparable to the best that can be obtained by the common alternative technique, powder XRD.[18–20] For detecting drug substances in formulated products, the detection limits of powder XRD are generally higher than we demonstrate herein by NMR because of interference by crystalline matrix components. For example, the powder XRD pattern of FFD in lactose would be dominated by lactose signals, and there is no XRD equivalent of dipolar dephasing to discriminate against them. Moreover, powder XRD fails for detection and quantification of low levels of amorphous materials.

We only examined an FFA sample contaminated with FFD. The dipolar-dephased spectrum (with TOSS) is illustrated in Figure 5a, and a suitable intensity of the analogous FFD spectrum was subtracted to give Figure 5b, which is therefore of FFA alone. It is clear that the FFA lines are somewhat broadened. This results in significant overlap with FFD signals in most cases. Thus, the formoterol fumarate system is not an ideal one for quantitation of crystal modification mixtures, which increases the significance of the results reported here. The FFA spectrum contains broad

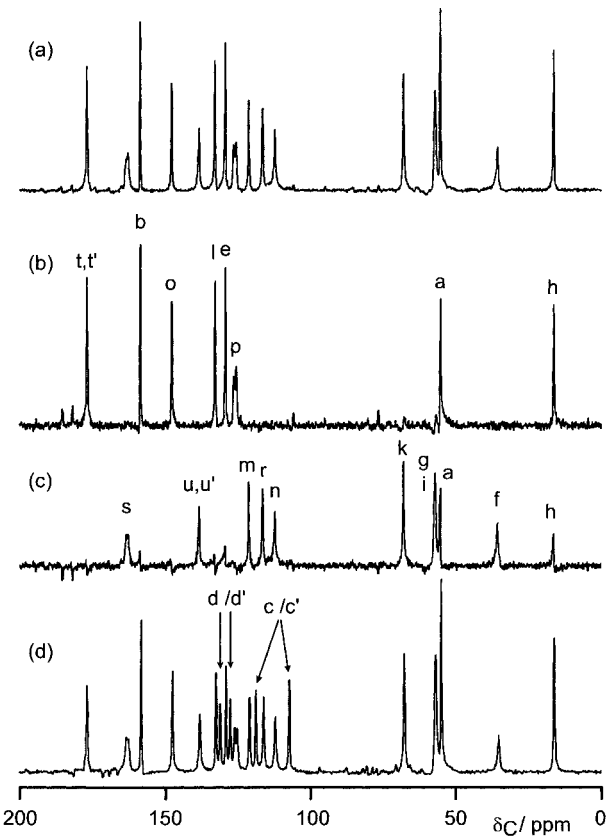



**Figure 3.** The 75-MHz $^{13}C$-{$^1H$} CPMAS spectra of FFD: (a) full spectrum obtained at ambient probe temperature, (b) dipolar dephased spectrum, (c) subspectrum of protonated carbons [obtained by suitable subtraction of (b) from (a)], and (d) full spectrum obtained at −50°C. The assignments are indicated on (b) and (c). The TOSS sequence was used. The number of transients accumulated was 696 for (a), 200 for (b), and 384 for (d). The spin rate was 4 kHz. The decoupling window for (b) was set to 40 μs.

lines at ca. 114 and 127 ppm, which we believe arise from the protonated phenylene carbons, perhaps affected by internal rotation (because they still appear to some extent in the dipolar dephased spectrum). The aliphatic region for FFA is significantly more complex than that for FFD. Most carbons seem to give rise to at least two resonances. There are a number of possible reasons for this. We believe the most likely is that the crystal structure is disordered with respect to the conformations of the chain linking the aromatic groups and of the methoxy group. Such a hypothesis would also, perhaps, explain the significant broadening of most of the aromatic signals (although this might also arise if crystallite sizes are very small). The chemical shifts for FFA are

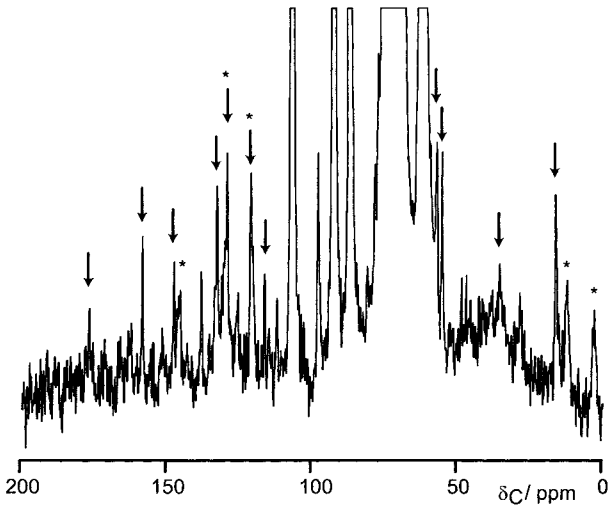



**Figure 4.** The 75-MHz $^{13}C$-{$^1H$} CPMAS spectrum of 0.45% FFD in lactose. The spin rate was 4.6 kHz and 18,000 transients were accumulated. TOSS was not used. Spinning sidebands are indicated by asterisks and FFD signals by vertical arrows.

listed in Table 1 as far as possible. The spectrum of FFA does not significantly change from ambient probe temperature down to −50°C.

The optimum distinction between the FFA and FFD spectra is for the fumarate carboxyl carbon

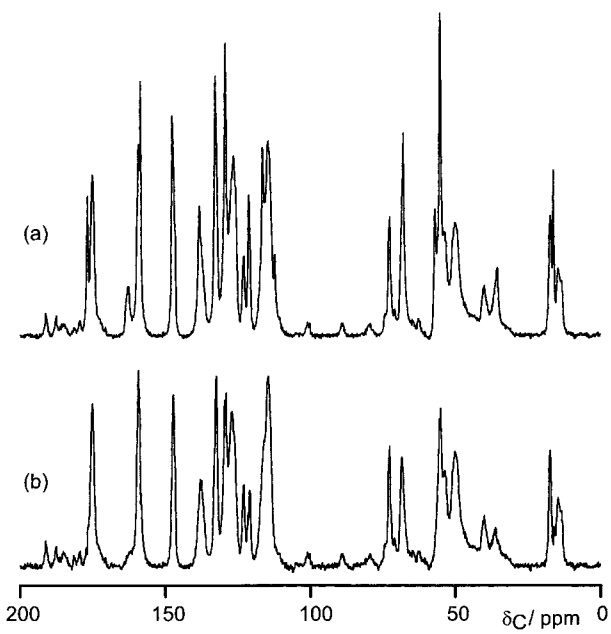



**Figure 5.** (a) Dipolar-dephased (with a 40-μs decoupling window) 75-MHz $^{13}C$-{$^1H$} CPMAS spectrum of FFA contaminated with FFD. The spin rate was 4480 Hz and 2000 transients were accumulated. (b) Spectrum (a) with that of a proportion of FFD subtracted.

**Table 2.** Cross-Polarization Properties of FFA/FFD

| Form (Signal) | $T_1$(H)/s | $T_{CH}$/μs | $T_{1\rho}$(H)/ms | S(0) | S(1 ms) |
|---|---|---|---|---|---|
| FFD (176.8) | 2.1 | 512 | 11.9 | 0.83 | 0.86 |
| FFA (175.2) | 1.4 | 510 | 13.9 | 1 | 1 |
| FFD + FFA (68.0) | 2.3 | 152 | 10.4 | 1 | 1 |
| FFA (72.8) | 1.6 | 118 | 11.3 | 0.57 | 0.57 |

signals at high frequency, presumably because the highly ordered FFD form exhibits stronger hydrogen bonding. This region was chosen for evaluation of quantitation for FFA/FFD mixtures. It would also be feasible to use signals at $\delta_C = 67.9$ and 72.6 ppm, but this is less desirable because, whereas the former arises from FFA alone, the latter is composed of an overlap of FFA and FFD resonances.

At all events, for any relative quantitation, it is essential to ensure comparability of CP efficiency, which can only be obtained if the CP rate, $T_{CH}$, and the proton relaxation times in the lattice and rotating frames ($T_1$ and $T_{1\rho}$, respectively) are measured. These were determined by pre-CP proton saturation recovery for $T_1$(H) and arrayed variable-contact-time CP for $T_{CH}$ and $T_{1\rho}$(H), using the FFA/FFD mixture to ensure the optimum compatibility with the quantitation experiments. The results for the four peaks of particular interest are given in Table 2.

To compare intensities for two different signals from a CP experiment, it is the values as functions of contact time, τ, extrapolated from long values to $\tau = 0$, namely, S(0), that should be used. In this case, however, for the pairs of signals listed, the ratio of the signals given by S(0) is the same (to within the error of measurement) as the ratio with a contact time of 1 ms. So, as far as the cross-polarization behavior is concerned, we can use the intensity data for these signals quantitatively. However, there does appear to be some difference in $T_1$(H) for FFD and FFA. With these $T_1$ values and using a recycle delay of 3 s in the CP experiment, we would expect to obtain 88% of the total available signal on each repetition for FFA and 76% for FFD. Therefore, under these conditions, the measured FFA content will be slightly higher relative to that of FFD than the true value, but corrections for this effect can be readily applied.

The dipolar dephasing experiment has fewer complications from residual sidebands and therefore provides the preferred approach, in particular making deconvolution easier. This is especially true for the signals at $\delta_C \sim 175$ ppm, which are optimum for quantitative analysis of FFA/FFD mixtures. Figure 6 shows an expansion of the relevant region of Figure 5a to illustrate the deconvolution into separate FFA and FFD signals. This gives a value for the anhydrate content of 77%. The result varies depending on exactly how the deconvolution is performed (whether Lorentzian or Gaussian lines are used), so that, given the differences in the $T_1$(H) values, the true figure is likely to be between 70% and 80%. It has been assumed that both signals behave in the same way in the dipolar dephasing experiment.

Figure 7 shows the spectrum from a formulation at the 2% (by mass) level of an FFA/FFD mixture in lactose. The aim of this experiment was to see

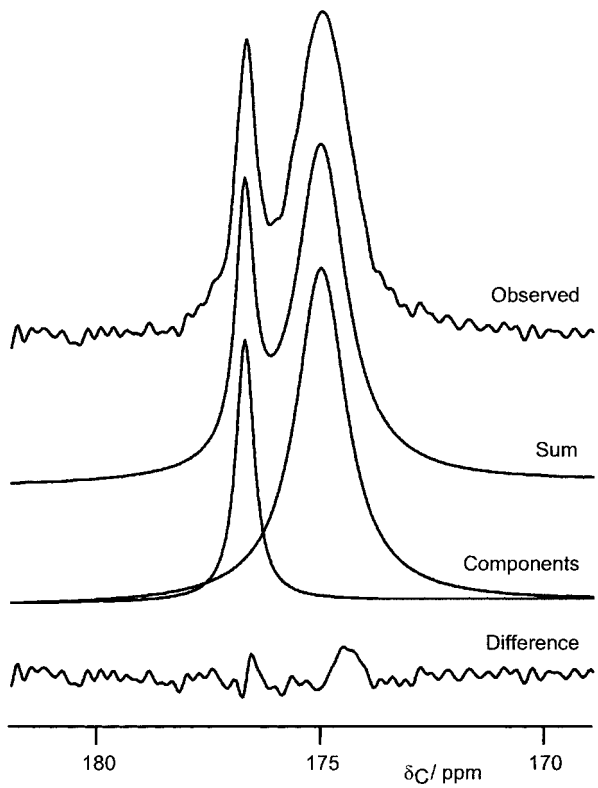



**Figure 6.** Expansion of the 175 ppm region of Figure 5a, to show deconvolution into FFD and FFA components.

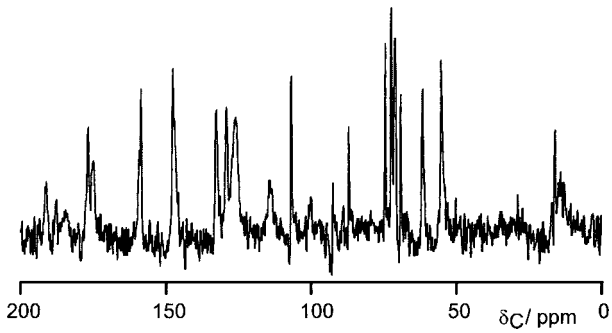



**Figure 7.** The 75-MHz $^{13}C$-{$^{1}H$} CPMAS spectrum of a 2% (by mass) mixture of FFA/FFD in lactose, using dipolar dephasing (with a 40-μs decoupling window). The spin rate was 4.6 kHz and 4 × 5000 transients were accumulated. The TOSS sequence was not used.

whether, at this concentration, the signals from both the FFA and FFD could be detected. Lactose does not contain any quaternary carbons, so the experiment chosen for Figure 7 involves dipolar dephasing, which minimizes the intensity of the lactose signals (careful adjustment of the dephasing delay might eliminate these completely but probably at the expense of some intensity in the FFA/FFD signals). It should also be noted that crystalline lactose has a complicated proton relaxation ($T_1$) profile (Apperley et al., unpublished results), which appears to depend on relative humidity. However, it is clear that the effective relaxation time is significantly longer than those of FFA and FFD, so a recycle delay of 3 s in CPMAS experiments further discriminates against lactose signals, which makes clear observation of FFA/FFD peaks easier. Amorphous lactose, however, has somewhat shorter relaxation times at $T_1$ = ca. 5 s, still longer than those of FFA and FFD. Initial assessment of Figure 7 suggested that the FFA/FFD ratio was different to that from the earlier spectra from the unformulated mixture. Although a usable result was produced after 4.25 h, the spectrum shown in Figure 7 was actually obtained from adding the free induction decays of four similar experiments (run consecutively). The sample was run this way to assess whether there were any changes during the experiment (there appear not to be). Deconvolution of the 175 ppm region, shown in Figure 8, reveals that the FFA/FFD ratio is 43%:57%, with accuracy estimated as ±5%. The difference in the FFA/FFD ratio (increased FFD) relative to that obtained from the earlier spectrum therefore appears to arise from the preparation of the sample before the experiment.

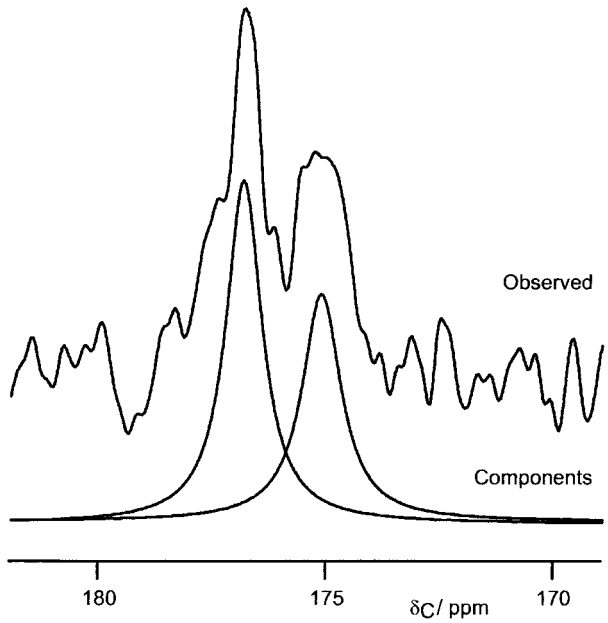



**Figure 8.** Expansion of the 175 ppm region of Figure 7, to show deconvolution into the FFD and FFA components.

## CONCLUSIONS

We have shown that formoterol fumarate dihydrate can be detected at a mass ratio of 0.45% in lactose by $^{13}C$ CPMAS NMR. Moreover, reliable concentration ratios of the dihydrate to the anhydrous form of formoterol fumarate can be obtained at a total 2% mass ratio in lactose. Complications from lactose signals can be minimized by using the dipolar dephasing pulse sequence and/or the TOSS sequence and/or recycling delays of ca. 3 s (i.e., between the longitudinal relaxation times of the target molecules and the excipient). However, these techniques may reduce the sensitivity of observation of the formoterol fumarate spectrum.

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