Solid-State Nuclear Magnetic Resonance (NMR) Spectra of Pharmaceutical Dosage Forms

Patricia J. Saindon,[1] Nina S. Cauchon,[1] Paul A. Sutton,[1] C.-j. Chang,[1] Garnet E. Peck,[1] and Stephen R. Byrn[1,2]

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Solid-state ^{13}C NMR spectra of tablets or capsules of prednisolone, enalapril maleate, lovastatin, simvastatin, ibuprofen, flurbiprofen, mefenamic acid, indomethacin, diflunisal, sulindac, and piroxicam were obtained in the CP/MAS mode at 50 MHz. These studies show that (1) solid-state NMR spectroscopy can detect the active ingredients in low-dose tablets and capsules; (2) the use of interrupted decoupling often results in suppression of resonances due to excipients, thereby allowing better detection of resonances from the drug; and (3) the technique permits discrimination between two prednisolone polymorphs present in tablets obtained from various manufacturers even though the tablets contain only approximately 5% (w/w) of the drug.

KEY WORDS: solid-state nuclear magnetic resonance; drugs; tablets; capsules; polymorphs.

INTRODUCTION

Solid-state ^{13}C NMR spectroscopy is a powerful method for the study of materials in the solid state. Using the combined techniques of high-power proton decoupling, cross-polarization (CP), and magic-angle spinning (MAS), high-resolution ^{13}C spectra can be obtained (1) which can give detailed atomic level information about the dynamics and local chemical environments. Over the past 10 years solid-state ^{13}C NMR has been used to study several pharmaceuticals including cefazolin, benoxaprofen, and nabilone (2), prednisolone *t*-butylacetate (3), enalapril maleate (4), carbamazepine (5), aspirin (6,7), and acetaminophen (8). Many of these papers show that different crystal forms have different solid-state NMR spectra (2–5). The study of carbamazepine (5) shows that solid-state NMR can be used to determine the amounts of the different hydrated crystal forms present in a bulk sample. It is of interest to the pharmaceutical industry to be able to characterize drugs directly in their final dosage forms. One hindrance to the characterization of drugs in final products is the presence of excipients. An important question which needs to be answered is: Can solid-state NMR detect small amounts of drugs in the presence of large amounts of excipients?

Surprisingly little research has been done on the solid-state NMR spectra of pharmaceutical dosage forms (6–8). To our knowledge, the only tablets to be investigated to date have been aspirin (6,7) and acetaminophen (8). The present paper reports solid-state ^{13}C NMR studies of tablets of prednisolone, enalapril maleate, lovastatin, and simvastatin, all of which contain relatively small amounts of the drug in the presence of large amounts of excipients. We also report the spectra of tablets or capsules of the nonsteroidal antiinflammatory agents ibuprofen, flurbiprofen, mefenamic acid, indomethacin, diflunisal, sulindac, and piroxicam.

The results presented herein show that solid-state NMR can detect drugs in the presence of large amounts of excipients, as in the case for prednisolone tablets which contain 5% (w/w) of the drug in the final dosage form. In addition, for prednisolone, our studies indicate that one of the commercially available dosage forms contained a different polymorph from the others. Finally, these studies show that solid-state NMR can be used to differentiate between tablets containing drug and tablets containing placebo in samples used for clinical trials. The overall conclusion from these investigations is that solid-state NMR spectroscopy is a powerful method for the study of pharmaceutical dosage forms.

MATERIALS AND METHODS

All of the tablets or capsules studied were obtained by purchase from the Purdue University Pharmacy, except for enalapril maleate, lovastatin, and simvastatin, which were provided by the manufacturer. The placebo tablets were also obtained from the manufacturer. Form I of prednisolone was crystallized from 2-propanol. Form II was crystallized from acetone.

Solid-State NMR Methods

The solid-state ^{13}C NMR measurements were made with a Chemagnetics M-200 FT NMR spectrometer operating at 199.6 MHz for ^{1}H and 50.2 MHz for ^{13}C. Tablets were lightly ground in a mortar and pestle and approximately 250 mg of material was packed into a bullet designed Kel-F or zirconium rotor. Capsules were emptied directly into the rotor and packed. High-resolution spectra were obtained using high-power proton decoupling and cross-polarization (CP) with magic angle spinning (MAS) at approximately 3–3.5 kHz. The parameters varied depending upon the sample. The contact time was usually 2 msec, and the pulse delay between scans was either 2 or 3 sec (see Table III). One thousand to forty thousand transients were collected, depending upon the sample, with spectral widths of approximately 15 kHz. The FIDs were processed using line broadening factors of 10–20 Hz. All chemical shifts were externally referenced to the methyl signal of hexamethylbenzene at 17.36 ppm. The interrupted-decoupling experiments were done using a 50-μsec delay without proton decoupling between the contact time and the data acquisition.

Crystallographic Determination of Unit Cell Parameters

Needlelike crystals of Forms I and II of prednisolone were positioned on a Siemens/Nicolet Diffractometer with their long axis at approximately 45° with respect to the axis

[1] Departments of Medicinal Chemistry and Pharmacognosy and Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana 47907.

[2] To whom correspondence should be addressed at Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907.

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Table I. Unit Cell Parameters for Forms I and II of Prednisolone

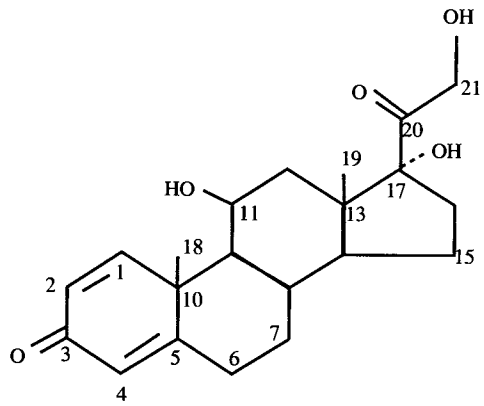
Crystal form	Z	a (Å)	b (Å)	c (Å)	β	Volume Å^3	Volume Per molecule (Å^3 per molecule)	Space Group
I	2	6.350 (3)	12.985 (8)	10.971 (9)	91.24 (5)	904	452	$P2_1$
II	4	11.808 (7)	6.009 (2)	25.643 (12)	90.0	1820	455	$P2_12_12_1$

of the goniometer, and cell constants were obtained from the least-squares analysis of the refined setting angles of 15 reflections.

Dissolution Testing

The sample prednisolone tablets considered in this study were evaluated by the USP XII/NF XVII using method 2. Five-milliliter samples were removed every 5 min until complete dissolution seemed apparent; i.e., no remaining tablet appeared in the flask. The medium was that recommended in the monograph for prednisolone.

Table II. Comparison of the Solid-State ^{13}C NMR Chemical Shifts (ppm) of the Two Crystal Forms of Prednisolone to the Solution Chemical Shifts[a]



Form I	Form II	Solution	Carbon atom
209.5	211.8	211.5	C(20)
188.1	187.9	185.1	C(3)
175.1	171.0	170.5	C(5)
159.8	157.3	156.8	C(1)
125.9	130.2	127.2	C(2)
121.8	123.8	121.7	C(4)
91.4	90.2	88.5	C(17)
69.9	70.4	68.6	C(11)
67.1	67.7	66.1	C(21)
55.4	54.8	55.5	C(9)
52.2	52.8	51.2	C(14)
47.5	47.1	46.7	C(13)
45.3	45.1	43.9	C(10)
42.1	43.1	39.0	C(12)
35.3	34.7	34.1	C(8)
34.3	33.5	33.0	C(16)
33.5	32.7	32.7	C(15)
31.8	31.5	31.6	C(6)
24.6	25.4	31.2	C(7)
23.9	23.7	21.0	C(18)
17.3	18.1	17.0	C(19)

[a] The peak assignments shown are based on the assignments in Ref. 9. The assignments for C(8), C(16), C(15), C(6), C(7), and C(19) are ambiguous and therefore tentative.

RESULTS AND DISCUSSION

Prednisolone

Prednisolone exists in at least three crystal forms. Table I reports the unit cell parameters for the two forms of greatest interest, Form I and Form II, the crystal structures of which will be reported separately. Table II reports the chemical shifts of these two forms along with tentative peak assignments based on the solution assignments (9). It is clear from these data that Forms I and II have distinctly different solid-state NMR spectra. The major chemical shift differences between the two forms are for C(2), which differs by 4.3 ppm, and C(5), which differs by 4.1 ppm.

Figure 1 shows the solid-state ^{13}C NMR spectra of two prednisolone products. It is clear from this figure that one product contains Form II, while the other contains Form I. This is most apparent by comparing the splitting between the peaks between 120 and 140 ppm as shown in the inset to Fig. 1. The spectrum of one product has these two peaks closer together than in the other product. This result clearly shows that solid-state NMR can be used to determine the polymorph present in a dosage form even if the drug composes only a small amount of the total weight of the tablet. In the case of these tablets the labeled amount is 5 mg per tablet and a tablet weighs approximately 100 mg.

Since one product contains a different polymorph from the other, the dissolution rates of these products were determined. Even though these products contain different crystal forms (a quality control issue?), their dissolution is the same within experimental error. This is not unusual, since these two crystal forms appear to have very similar packing density (volume per molecule in the crystal), 452 vs 455 Å^3/molecule (see Table I).

Lovastatin (Mevacor)

Lovastatin is an example of an HMG–CoA reductase inhibitor which is effective as a cholesterol lowering agent. The solid-state ^{13}C NMR assignments are shown in Table IV along with the solution assignments (10). Figure 2 shows the solid-state NMR spectrum of two lovastatin tablets, which each have labeled amounts of 20 mg. The excipients obscure resonances which appear at the same chemical shift but have little effect on resonances at other chemical shifts. Similar

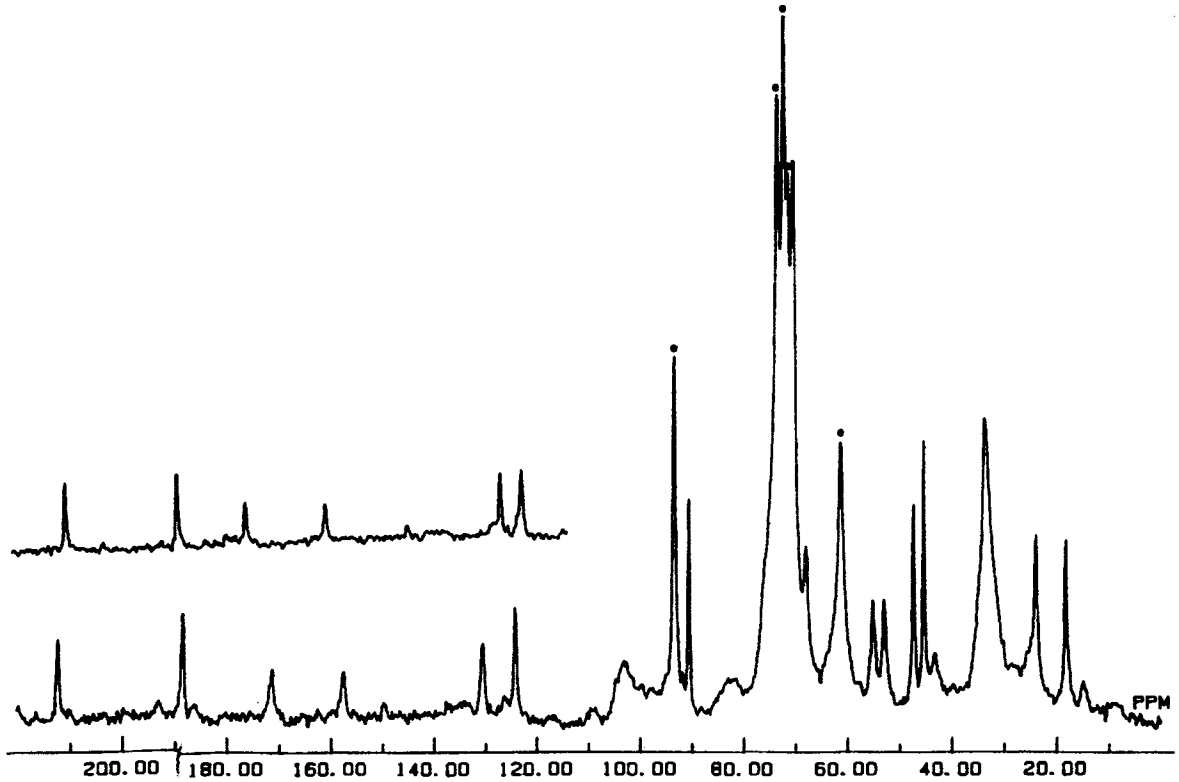


Fig. 1. Solid-state CP/MAS spectra of prednisolone tablets from two manufacturers. The inset shows the region of greatest difference between Form I and Form II of prednisolone (see Table II) and shows that this product contains prednisolone in Form I. The signals from the excipients are marked.

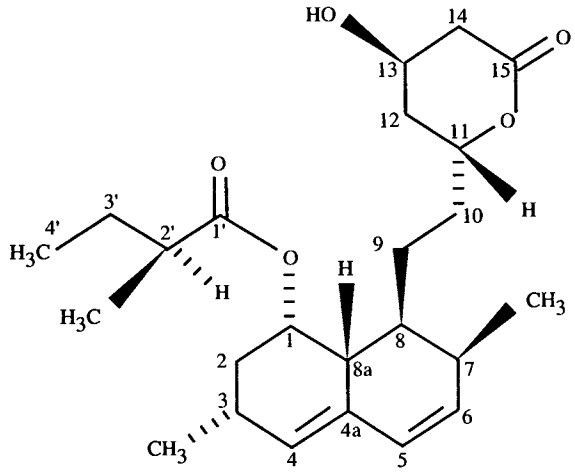
Table III. Tablets and Capsules of Drugs Investigated in this Study by Solid-State ^{13}C NMR[a]

Drug	Dose (mg)	Approximate weight of dosage form (mg)	Manufacturer and trade name	Number of scans
Prednisolone tablets	5	100		
		110		
		95		
Enalapril maleate tablets	20	200	Merck, Sharp, and Dohme (Vasotec)	16,000 (33,576)[b] each
	5	230		
	2.5	—		
	1.25	230		
Lovastatin tablets	20	—	Merck, Sharp, and Dohme (Mevacor)	—
Simvastatin tablets	40	400	Merck, Sharp, and Dohme	4,000 (4,000)
Ibuprofen tablets	200	320	Bristol-Meyers Squibb (Nuprin)	2,000 (4,048)
	200	330	Upjohn (Haltran)	7,264 (2,692)
	400	620	Geneva Generics	6,000 (9,084)
Sulindac tablets	200	330	Merck, Sharp, and Dohme (Clinoril)	3,000 (8,104)
Flurbiprofen tablets	100	420	Upjohn (Ansaid)	4,000 (10,000)
Diflunisal tablets	500	840	Merck, Sharp, and Dohme (Dolobid)	10,000 (20,492)
Indomethacin capsules	50	350	Merck, Sharp, and Dohme (Indocin)	12,000 (20,000)
	75	280	Merck, Sharp, and Dohme (Indocin-SR)	12,000 (28,248)
	50	480	United Research Laboratories	10,000 (30,000)
	50	380	Geneva Generics	10,000 (27,772)
Mefenamic acid capsules	250	350	Parke-Davis (Ponstel)	7,000 (6,000)
Piroxicam capsules	20	300	Pfizer (Feldene)	14,000 (19,556)

[a] The contact time used in each case was 2 msec. The delay times were 3 sec for the prednisolone tablets and 2 sec for the other tablets and capsules.

[b] The number in parentheses refers to the interrupted-decoupled spectra.

Table IV. Solid-State NMR Chemical Shift Assignments for Lovastatin (ppm)



Solid state	Assignment	Solution in $CDCl_3$
179.1	1′	176.9
170.9	15	170.6
135.9	6	133.0
134.2	4a	131.5
128.2	4	129.5
128.2	5	128.4
76.5	11	76.4
69.3	1	67.9
62.3	13	62.4
40.9	2′	41.5
38.9	14	38.6
37.1	8a	37.3
34.1	8	36.6
34.1	12	36.0
33.6	10	32.9
33.6	2	32.6
29.9	7	30.7
29.9	3	27.4
28.1	3′	26.8
24.3	9	24.2
23.4	3-CH_3	22.8
13.7	2′-CH_3	16.2
13.4	7-CH_3	13.8
12.4	4′	11.7

results were obtained for tablets of another closely related HMG–CoA reductase inhibitor, simvastatin (see Table III).

Enalapril Maleate (Vasotec)

The spectra of the tablets containing different labeled amounts of enalapril maleate were measured under identical conditions. As might be expected the 20-mg tablets showed significantly stronger signals than the other tablets (5, 2, and 1.25 mg). The peaks at 175 ppm are carbonyl resonances due to drug and maleate salt. Figure 3 shows the CP/MAS spectrum of two 20-mg tablets. Comparisons with the published spectrum (4) and the placebo spectrum show that the large peaks in the 50- to 120-ppm range are due to excipients. The small peaks at 14.5, 129.9, and 175.8 ppm are due to the enalapril maleate and are consistent with the spectra shown by Ip *et al.* (4). In contrast to prednisolone, the resonances are not sharp enough to allow a determination of the polymorph present.

Interrupted decoupling is a useful method for analysis and signal assignment in solid-state ^{13}C NMR spectra (11). A brief delay (typically 50 μsec) is inserted between the end of the ^1H–^{13}C contact time and the data acquisition with proton decoupling. During this period, the magnetization due to carbon atoms that are directly attached to protons rapidly dephases, with the exception of the methyl groups, in which rapid internal motion averages out the ^{13}C–^1H dipolar interactions. The resulting spectrum therefore shows only signals due to quarternary and methyl carbons. The interrupted-decoupled spectrum of the 20-mg tablets (Fig. 4) shows two peaks in the methyl region, the 63.1-ppm peak, and two peaks in the 177- to 185-ppm range. The interrupted-decoupled spectrum of the placebo tablet shows only two very small peaks, at 15.96 and 63.20 ppm. Therefore, interrupted decoupling provides a convenient and powerful method to suppress peaks due to the excipients, which frequently have a number of methylene and methine carbons. Hence, the resulting spectra often show only peaks that are due to the methyl and quarternary carbons in the active ingredient, thus facilitating their observation and assignment. We have also found this to be the case in other studies of tablets and capsules.

The CP/MAS spectra of 5-mg enalapril maleate tablets were measured with the same number of scans as the 20-mg tablet. The peaks in this spectrum (not shown) are significantly smaller than those from the 20-mg tablets. This is no doubt because of the smaller amounts of enalapril maleate in these tablets. The interrupted-decoupled spectra of the 5-mg tablets are consistent with this result. In the interrupted-decoupled spectrum, only the peak at 177.2 ppm is discernible, in contrast to those observed in the 20-mg tablets (see Fig. 4). The peak at 16.2 ppm is assumed to be identical to that of the placebo which appears at 16.0 ppm. Since the same number of scans were used for both the 20- and the 5-mg tablets, again the relative intensity of the solid-state NMR signals is consistent with the amounts in two tablets. The spectra of the 2.5-mg tablets are similar to those of the 5-mg tablets. The spectra of the 1.25-mg tablets (not shown) are virtually identical to the spectrum of the placebo. Thus, CP/MAS ^{13}C NMR is not sensitive enough to detect the drug in this formulation.

In conclusion, it is clear that the intensity of the peaks is consistent with the amount of enalapril maleate present. However, the peaks are significantly broader than in the case of prednisolone. This may be due to the more amorphous nature of the material. Observation of the signals due to the active ingredient in a solid dosage form may thus depend not only on the labeled amount of the drug but also on its degree of crystallinity.

Nonsteroidal Antiinflammatory Agents

The solid-state ^{13}C NMR spectra of a number of tablets and capsules of various nonsteroidal antiinflammatory agents were also obtained (Table III). As expected from our previous results, the spectra of the ibuprofen tablets showed

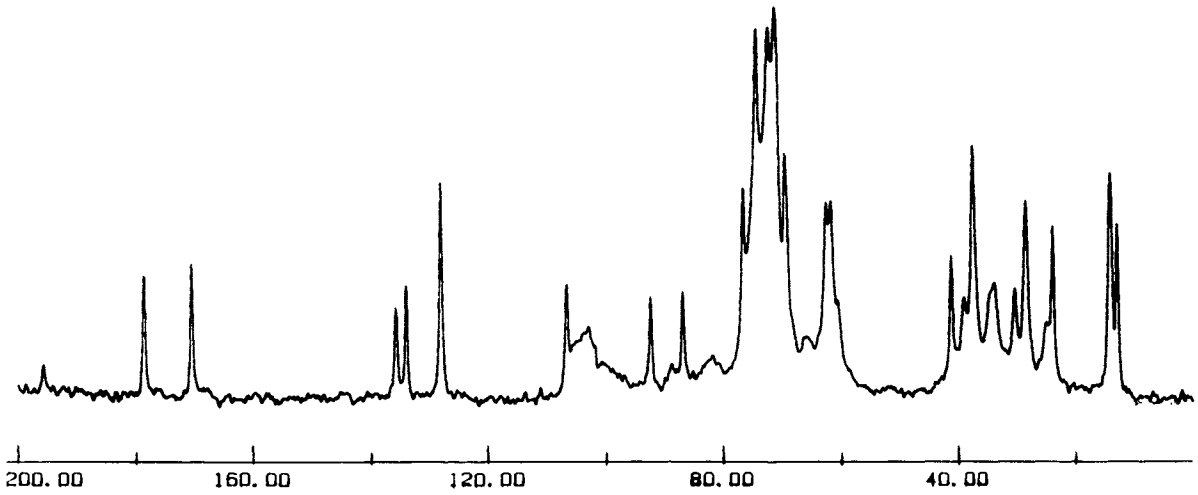


Fig. 2. Solid-state CP/MAS spectrum of two 20-mg tablets of lovastatin.

narrow lines with excellent signal-to-noise ratios, since the drug itself is crystalline and is present in a high dose in the tablets (see Fig. 5 and Table V). In general, most of the resonances due to the drugs were clearly distinguishable in the spectra of the dosage forms and could be assigned based on the published solution ^{13}C NMR assignments (12–16). The interrupted-decoupling pulse sequence was again used to simplify the appearance of the spectra by suppressing excipient peaks and to aid in signal assignment. As with the other studies reported here, it is clear that solid-state NMR is a powerful and useful tool for the study of pharmaceutical solids and their dosage forms.

In conclusion, this study shows that solid-state ^{13}C NMR spectroscopy can be used to determine the crystal form present in low-dose tablets and to identify whether a tablet is a placebo or contains the drug. Although we have

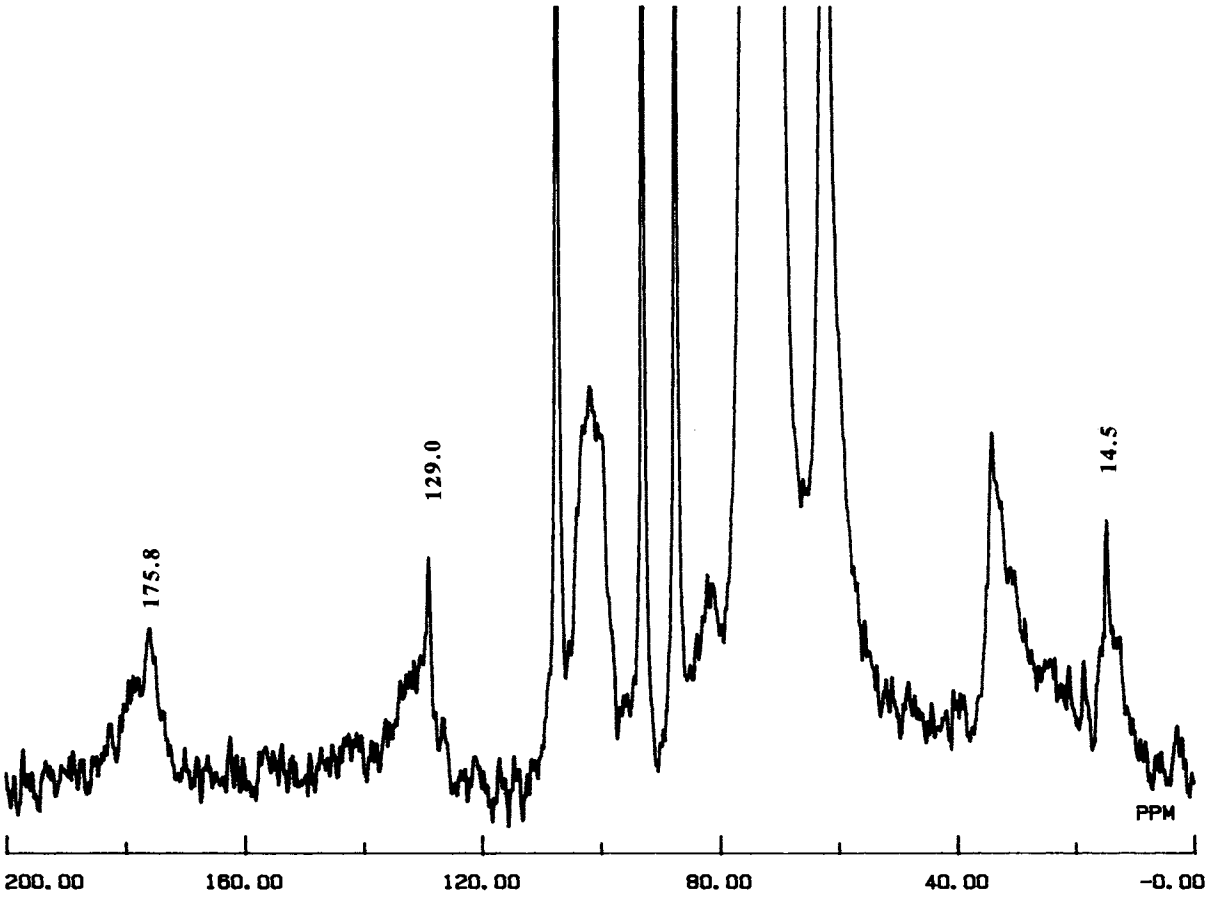


Fig. 3. Solid-state CP/MAS spectrum of approximately two 20-mg tablets of enalapril maleate.

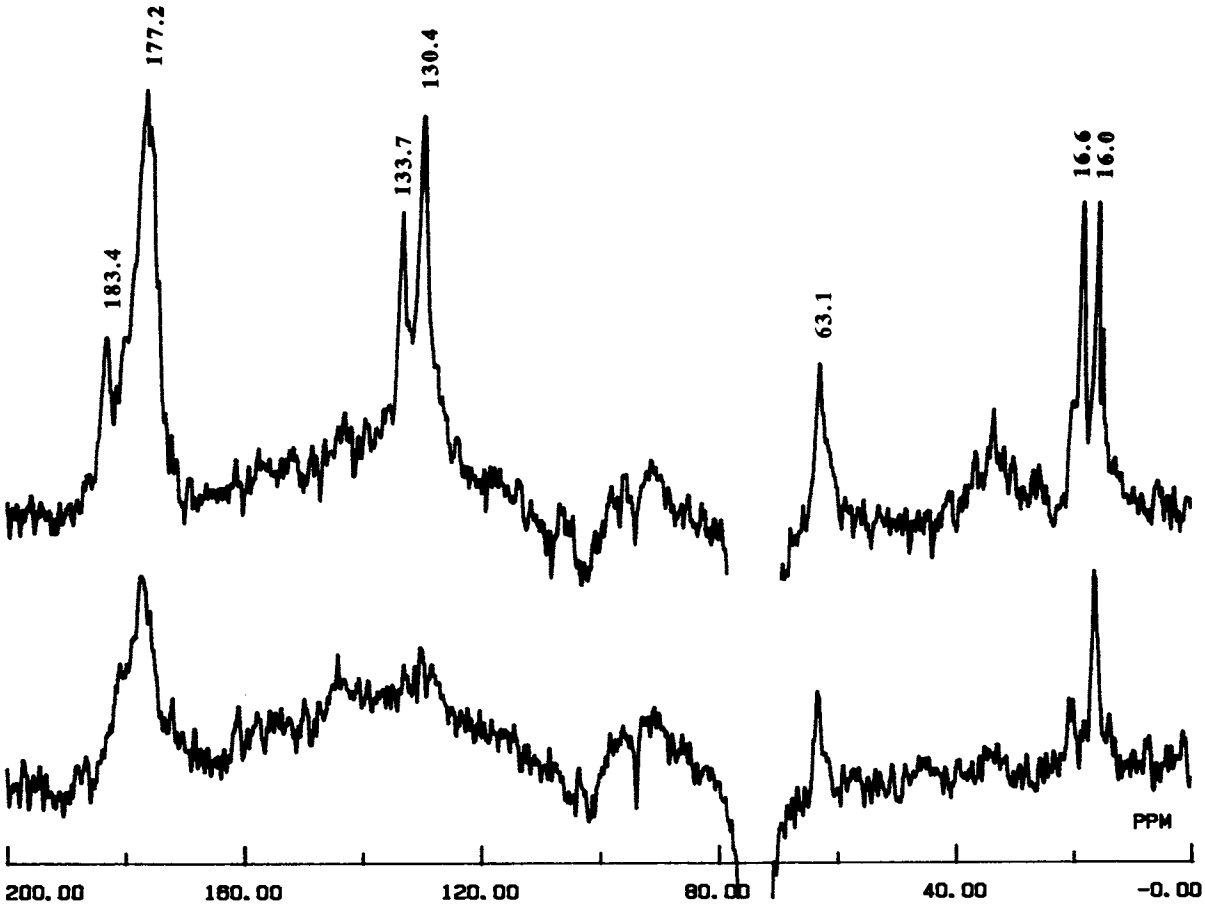


Fig. 4. Solid-state CP/MAS interrupted-decoupled spectrum of approximately two 20-mg tablets of enalapril maleate (top) and of two 5-mg tablets of enalapril maleate (bottom).

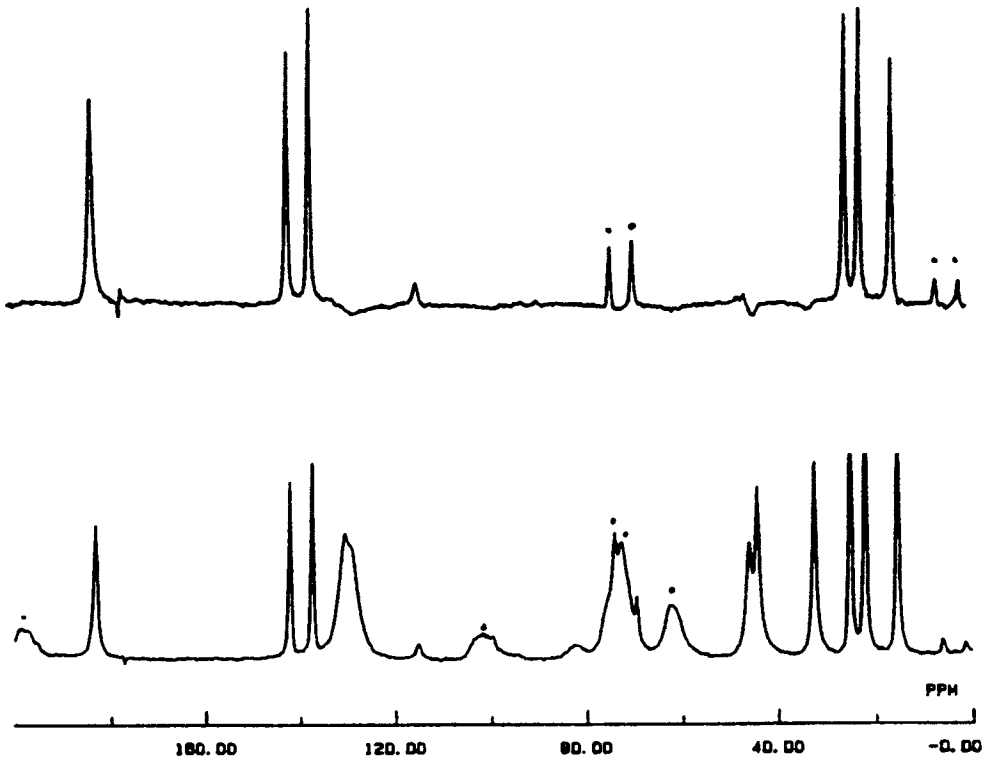
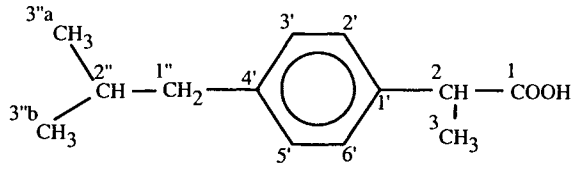


Fig. 5. Spectra of 200-mg ibuprofen tablets (Nuprin). The top spectrum is the interrupted decoupling spectrum and the bottom spectrum is the CP/MAS spectrum. The excipients, or spinning side bands, are marked with a dot.

Table V. Solid-State Chemical Shift Assignments for Ibuprofen



C	δ (ppm) Solid state	Solution ($CDCl_3$)
1	183.3	181.5
4'	142.1	140.7
1'	137.3	137.1
3',5'	130.9	129.4
2',6'	129.3	127.4
	127.1	
1",2	46.0	45.1
	44.2	
2"	32.7	30.2
3"a,b	25.1	22.4
	22.1	
3	15.4	18.7

not determined the X-ray powder diffraction patterns of the low-dose tablets studied here, we believe that solid-state NMR offers a useful alternative for direct determination of the crystal form present in the final dosage form.

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