Polymorphic Characterization of Pharmaceutical Solids, Donepezil Hydrochloride, by ^{13}C CP/MAS Solid-State Nuclear Magnetic Resonance Spectroscopy

Tae-Joon Park, Dong-Hyun Ko,† Young Ju Kim,† and Yongae Kim*

Dept. of Chemistry and Protein Res. Cener for Bio-Industry, Hankuk University of Foreign Studies, Yongin 449-791, Korea
**E-mail: yakim@hufs.ac.kr*
†CJ Cheil Jedang Corp. Pharmarceutical Research Institute, Icheon 467-812, Korea
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Donepezil hydrochloride is a reversible acetylcholinesterase inhibitor that is used in the treatment of Alzheimer's disease to improve the cognitive performance. It shows different crystalline forms including hydrates. Therefore, it is very important to confirm the polymorphic forms in the formulations of pharmaceutical materials because polymorphs of the same drug often exhibit significant differences in solubility, bioavailability, processability and physical/chemical stability. In this paper, four different forms of donepezil hydrochloride were prepared and characterized using X-ray powder diffraction, Fourier transform infrared, and solid-state nuclear magnetic resonance (NMR) spectroscopy. This study showed that solid-state NMR spectroscopy is a powerful technique for obtaining structural information and the polymorphology of pharmaceutical solids.

Key Words: Donepezil, Pharmaceutical solids, Solid-state NMR, CP/MAS, Polymorphism

Introduction

In recent years, the solid-state properties of drugs has attracted a great deal of attention in the pharmaceutical industry, as a major contributing factor to both bio-availability and formulation characteristics.[1-7] Most pharmaceutical drugs are solids and exist in many different physical forms, including amorphous, polymorphic crystalline or hydrated forms. The ability of some substances to exist in more than one crystalline form, known as polymorphism, is one of the most important solid-state properties of drugs. While polymorphs have the same chemical composition, they differ in their packing and geometrical arrangement, which can affect the pharmaceutically important chemical and physical properties, such as melting point, chemical reactivity, solubility, dissolution rate, powder flow and tableting behavior.[8-12] Therefore, different crystalline forms of a pharmaceutically useful compound provide opportunities to improve the performance characteristics of a pharmaceutical product. Accordingly, it is necessary to verify the polymorphism and detect any polymorphic changes in drugs. Recently, the Food and Drug Administration (FDA) in US recognized the importance of polymorphs, and required that appropriate analytical procedures be used in drug guidelines to detect polymorphic, hydrated or amorphous forms of a drug.

There are a number of methods for characterizing the solid-state properties of pharmaceutical solids, such as optical and electron microscopy, X-ray analysis, infrared (IR) and Raman spectroscopy, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). Thermal methods, such as TGA or DSC, have limited accuracy due to the small weight fraction of the mobile solvent. Single-crystal X-ray analysis is generally used to understand the crystal packing of individual molecules within a crystal lattice. However, X-ray crystallographic analysis cannot usually detect solvent molecules, which are essential for growing high-quality single crystals of sufficient size as a meta-stable form. In addition, most of these techniques are unsuitable for studying amorphous compounds.

Solid-state NMR spectroscopy is a powerful technique for analyzing the structural, chemical and physical properties of pharmaceutical solids.[13] This technique is usually non-destructive and non-invasive. In addition, the samples to be analyzed require no special preparation. Compared to other solid-state characterization techniques, solid-state NMR spectroscopy provides more vital information on the local environment in crystal packing and mobility in pharmaceutical solids.[14] In practice, solid-state NMR spectroscopy is used widely to characterize and identify polymorphs, pseudopolymorphs with different hydrate and amorphous compounds.

Donepezil hydrochloride, 2,3-dihydro-5,6-dimethoxy-2-((2-(phenylmethyl)-4-piperidinyl)methyl)-1*H*-inden-1-one hydrochloride, inhibits acetylcholinesterase (Figure 1), and is used as a pharmaceutically active agent for the symptomatic treatment of mild to moderate Alzheimer's dementia, which is related to a cholinergic deficiency in the cortex and basal forebrain.[15-17] Donepezil hydrochloride has different crystalline forms, including hydrates.[18] Form III is thermodynamically stable, and form I is stable only at lower relative humidity because it absorbs additional water at higher relative humidity.[19]

In this study, crystalline form I and III of donepezil hydrochloride were synthesized, and their structural information and polymorphology were obtained. Interestingly, the synthesized crystalline form I has different hydrate forms, which are called a pseudo-polymorphic forms. Crystalline form I of donepezil hydrochloride, which has a 1.5% water content (form I-A), absorbs water slowly from the atmosphere to reach a water content of 5 - 6%, which is called form I-B. Form I-C is obtained by drying form I-B for 12 hrs at 70 ^{o}C in a vacuum to a residual water content of < 1%. The structural changes after hydration and dehydration from hydrated form I-A were

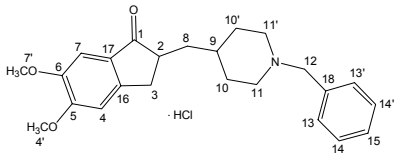

Figure 1. Chemical structure of donepezil hydrochloride.

also measured. Three different types of hydrate form I were characterized and compared using X-ray powder diffraction (XRD), Fourier transform infrared (FT-IR), and solid-state nuclear magnetic resonance (NMR) spectroscopy. In particular, we report a clearly different crystalline structure of synthesized donepezil hydrochloride from the ^{13}C cross-polarization/ magic-angle spinning (CP/MAS) solid-state NMR spectra.

Experimental Methods

Preparation of Crystalline Form I-A. 10 g of donepezil hydrochloride was suspended in 100 mL of methanol. The suspension was heated to 60 °C under reflux to dissolve the solids. The solution was then cooled to 10 °C, and 300 mL of tert-butyl methyl ether was added. The mixture was then stirred for an additional 1 hr at 10 °C, filtered, and dried in a vacuum at 70 °C to give form I-A of donepezil hydrochloride. The final yield was 9.3 g and the water content was 1.5%.

Preparation of Crystalline Form I-B. Form I-A crystal absorbs water slowly from the atmosphere to reach a water content of 5 - 6%, which is called form I-B.

Preparation of Crystalline Form I-C. Form I-C was obtained by drying form I-B for 12 hrs at 70 °C in a vacuum to a residual water content < 1%. The water content was determined using the Karl-Fischer titrimetric method. The Karl-Fischer titration was performed by charging 100 mg of the solid into a titration vessel using a DL38 Karl-Fisher Titrater (Mettler-Toledo, Switzerland).

Preparation of Crystalline Form III. 10 g of donepezil hydrochloride was suspended in 100 mL of methanol. The suspension was heated to 60 °C under reflux to dissolve the solids. 300 mL of tert-butyl methyl ether was added to the solution and stirred at room temperature for an additional 1 hr. The crystalline product was filtered and dried in a vacuum at 70 °C to give form III of donepezil hydrochloride. The final yield was 9.5 g and the water content was 0.2%. The content of water was determined using the Karl-Fischer titrimetric method.

FT-IR Spectroscopy. FT-IR spectroscopy was carried out using an IFS-66 spectrometer (Bruker, Germany). The IR spectra of the sample were obtained using a KBr pellet that was prepared with a press after careful grinding of a small amount of each sample with KBr. The spectral width was 400 - 4000 cm^{-1} and the spectral resolution was 4 cm^{-1}. Each spectrum was acquired by performing 32 scans.

X-ray Diffraction. The powder XRD patterns were recorded using an X'Pert PRO Diffractometer (PANalytical, The Netherlands). The samples were mounted on 0.2 mm cavity sample holders. A typical continuous scan was performed under the following conditions; scan type: standard, Axis: 2θ/θ, Start: 5 °, Stop: 40 °, Scan speed: 5 °/min, Sampling interval: 0.03, Data type: counts/sec, Voltage: 40 kV, Current: 30 mA.

Solid-State NMR Spectroscopy. The solid-state ^{13}C NMR spectra were obtained using a unity*INOVA* wide bore NMR spectrometer (Varian, USA) with a magnetic field of 9.4 Tesla operating at a ^{13}C Larmor frequency of 104.21 MHz. The ^{13}C NMR experiments were carried out using a 4-mm triple resonance CP/MAS probe (T3 probe Varian, USA). All spectra were obtained using a combination of the CP/MAS and total sideband suppression (TOSS) methods (CP/MAS/TOSS). In the $^{1}H/^{13}C$ CP/MAS/TOSS experiments, the Hartmann–Hahn polarization transfer with a contact time of 2 ms was optimized to operate at B_1 field strengths of 59.5 kHz and 40.0 kHz for the ^{1}H and ^{13}C channels, respectively.[20-23] Two-pulse phase-modulation (TPPM) proton decoupling was used to improve the proton decoupling with the two pulses each with a flip angle of 10.0 μs and a phase difference of 15° between pulses.[24]

All samples were ground softly with a mortar and pestle and then packed in a 4-mm zirconium oxide rotor. All measurements were taken at an ambient temperature of 20 to 24 °C. The magic angle was adjusted using the ^{79}Br resonance of KBr, and all CP/MAS/forTOSS measurements were carried out at a MAS frequency of 6.5 kHz. The ^{13}C chemical shifts were referenced externally to the carboxyl signal of glycine at 176.03 ppm.

Results and Discussion

FT-IR Spectroscopy. Figure 2 shows the FT-IR spectra of form I-A, I-B, I-C and III. Their overall patterns in the finger print region were similar. The most intensive absorption band around 1690 cm^{-1} in the spectra is attributed to the stretching vibrations of C=O group in the structure of donepezil hydrochloride. The absorption frequency of C=O group in anhydrous form III (1696 cm^{-1}) is some higher than in hydrate form I-A, I-B, and I-C (1684 cm^{-1}). Sharp absorption bands at around 3588 cm^{-1} are contributed by free O-H groups and broad absorption bands around 3900 cm^{-1} are contributed by bonded O-H groups of water molecules in the structure of hydrates in form I-A, I-B, and I-C. On the other hand, form III appears to be an

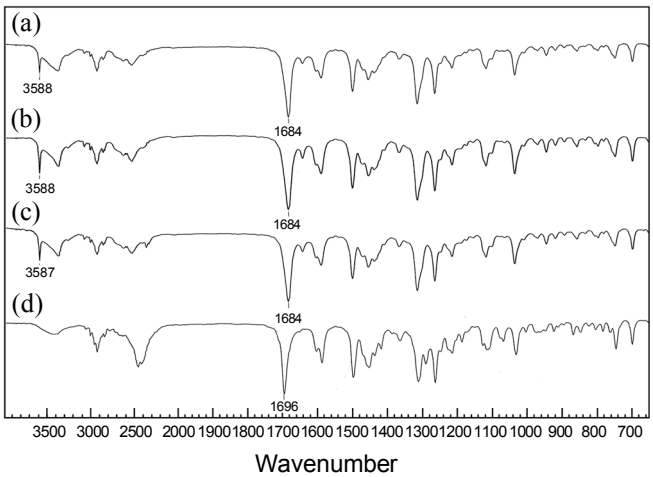

Figure 2. FT-IR spectra of form (a) I-A, (b) I-B, (c) I-C, and (d) III of donepezil hydrochloride.

anhydrous form because IR spectrum in Figure 2(d) shows no particular peaks in the O-H band region. Additionally, a difference between hydrates and anhydrous form is the presence of absorption band at 1640 cm^{-1}. It is probably because carbonyl groups are forming hydrogen bonds to water molecules. However, differences between the hydrate form I-A, I-B, and I-C were not prominent from the IR spectra.

Powder X-ray Diffraction. Figure 3 shows the powder XRD patterns of form I-A (a), I-B (b), I-C (c), and III (d). All the powder patterns contained sharp peaks indicating good crystallinity with identical 2θ values except for form III. An analysis of the conformation from the powder XRD data indicated that form I-A, I-B and I-C have mostly an identical conformation. However, form III had a different crystalline form with respect to form I, which is similar to the IR spectra.

Solid-State NMR Spectroscopy. Figure 4 shows the ^{13}C CP/MAS/TOSS solid-state NMR spectra of form I-A (a), I-B (b), I-C (c) and III (d) of donepezil hydrochloride. The spectra can be divided into five main regions. The chemical shifts from 200 - 210 ppm, 130 - 160 ppm, 100 - 130 ppm, 40 - 60 ppm and 10 - 40 ppm were respectively assigned to carbonyl groups, aromatic quaternary carbons, aromatic carbons, sp3-hybridized carbons bonded to either a nitrogen or an oxygen (e.g. piperidinyl or methoxy carbons), and sp3-hybridized carbons attached to carbon and/or hydrogen. The resulting spectra of the different forms of donepezil hydrochloride showed similar patterns, but with noticeably different peaks. Like the results of FT-IR spectroscopy and powder XRD, the ^{13}C solid-state NMR spectra in Figure 4 show a clear difference between crystalline form I and III. The spectrum of form III in Figure 4(d) showed resonances with different chemical shifts relative to the resonances of form I-A, I-B and I-C, and showed extremely different carbonyl carbon and methoxy carbon resonances. The chemical shift in solid-state ^{13}C NMR spectra reflects the local conformational distribution of molecules with respect to the applied magnetic field. Therefore, it is proposed that the crystalline form I and III have different crystal packing in the unit cell and a different morphology. The ^{13}C solid-state NMR spectra of form I in Figure 4(a), (b), and (c) show some different resonances, which is in contrast to the IR spectra and powder XRD pattern. Despite the slight difference in water content between form I-A (1.5%) and I-B (5 - 6%), the spectrum of form I-A in Figure 4(a) showed relatively broad and split resonances, particularly in the 120 - 140 ppm region from the aromatic car-

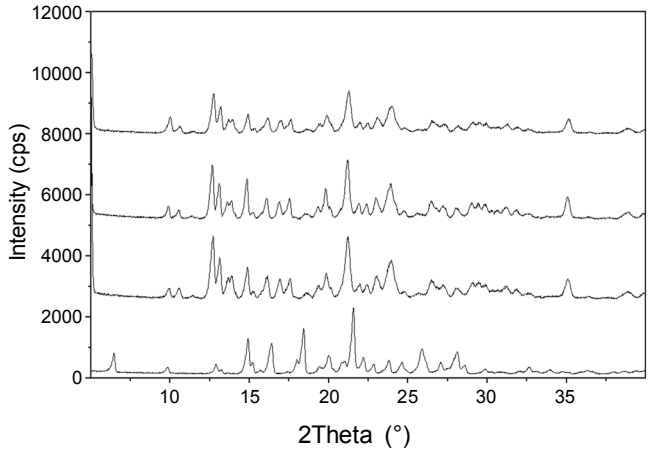

Figure 3. XRD patterns of form (a) I-A, (b) I-B, (c) I-C, and (d) III of donepezil hydrochloride.

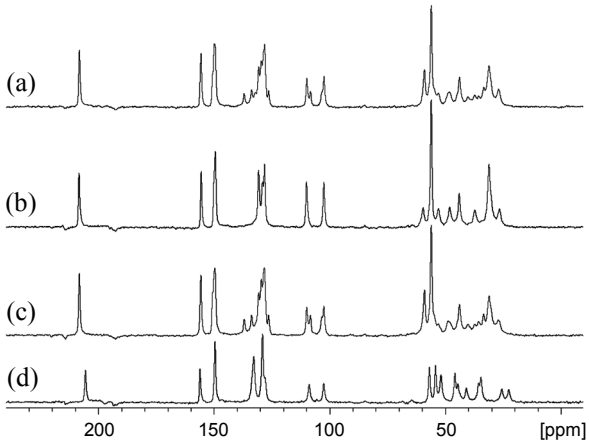

Figure 4. ^{13}C CP/MAS/TOSS solid-state NMR spectra of form (a) I-A, (b) I-B, (c) I-C, and (d) III of donepezil hydrochloride. The chemical shifts are in units of ppm referenced to the glycine carboxyl resonance at 176.03 ppm.

Table 1. ^{13}C resonance assignments of the CP/MAS/TOSS solid-state NMR spectra from Figure 4

(a) Form I-A	(b) Form I-B	(c) Form I-C	(d) Form III	Assignment
22.0 - 63.0	22.0 - 63.0	22.0 - 63.0	20.0 - 59.0	C9, C3, C10, C10', C8, C11, C11', C2, C4', C7', C12
102.3, 103.2	102.3	102.3, 103.2	102.4	C4
108.1, 109.7	109.8	108.1, 109.7	108.7	C7
124.0 - 140.0	125.0 - 129.6	124.0 - 140.0	126.0 - 130.5	C15, C14, C14', C13, C13'
	130.6		132.6	C16, C17, C18
149.7	149.3	149.7	150.0	C6
155.4	155.3	155.4	155.9	C5
208.0	208.2	208.0	205.4	C1

bons of phenylmethyl parts in the molecular structure. The spectrum of form I-C (< 1%), which is the dehydrated structure of form I-B, was similar to that of form I-A. However, there were no chemical shift differences in the carbonyl carbon and methoxy carbon resonances between form I-A, I-B and I-C. This suggests that form I-A, I-B and I-C have similar crystallographic packing but with a partially different molecular conformation, known as a pseudo-polymorph, which might be due to the residual water molecules incorporated into the crystal forms. Table 1 shows the ^{13}C chemical shift assignments of all carbon atoms in form I-A, I-B, I-C, and III of donepezil hydrochloride.

Conclusions

Four different forms of donepezil hydrochloride were prepared and characterized by FT-IR, XRD, and solid-state NMR spectroscopy. In this study, there were few molecular structural differences between the pseudo-polymorphs of form I. ^{13}C solid-state NMR spectroscopy provided useful information on the polymorphism of different crystalline packing and pseudo-polymorphisms of donepezil hydrochloride with different water contents in the solid state. These observations highlight the need for a detailed study of the relationship between hydrated donepezil hydrochloride and its crystalline packing.

Solid-state NMR analysis of different morphologies and pseudo-polymorphologies of pharmaceutical solids can provide important information that can often explain the conflicting results obtained with other analyses, such as IR, XRD, and thermal analysis. Overall, ^{13}C solid-state NMR spectroscopy can be a powerful tool for estimating the polymorphic and pseudo-polymorphic forms according to the different crystalline packing of molecules in a unit cell and different water-contents in a crystalline form.

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