

Characterization and Quantitation of Clarithromycin Polymorphs by Powder X-Ray Diffractometry and Solid-State NMR Spectroscopy

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Characterization of clarithromycin polymorph was performed by solid-state cross polarization and magic angle spinning (CP/MAS) ¹³C-NMR spectroscopy. Two polymorphs, form II and form I, of clarithromycins indicated characteristic resonances of C1 carbonyl carbon at 176.2 and 175.2 ppm, respectively. Since each peak of C1 carbon was well separated in the spectrum of the two polymorphs, we performed quantitative analysis of the polymorphic fraction from the peak area of these peaks. The peak area of form I was found to linearly increase with an increase of its content, with a correlation coefficient of above 0.99. Solid-state NMR was found to be a useful technique to determine the characteristics of the polymorphic forms.

Key words solid-state NMR; clarithromycin; polymorph; quantitative analysis

Polymorphs are major issue in the pharmaceutical industry, since different polymorphs show different stability and/or bioavailability.^{1,2)} In order to control polymorphism in the manufacturing process of medicines, methods must be developed to differentiate and quantitate different crystal forms. Solid-state ¹³C-NMR spectroscopy is a useful method for the study of materials in the solid state.³⁾ The combined techniques of cross polarization (CP) and magic angle spinning (MAS) give high-resolution ¹³C spectra, providing molecular level information.⁴⁾ Since the chemical shift was sensitive to molecular conformation and chemical environment in the crystal structure,⁵⁾ solid-state ¹³C-NMR spectroscopy has been developed for the pharmaceutical field.⁶⁻⁹⁾

In this study, we used clarithromycin, a 14-membered ring macrolide antibiotic which shows several polymorphic forms.¹⁰⁾ The polymorphs of the clarithromycins were characterized by solid-state ¹³C-NMR spectroscopy using the CP/MAS method. The application of solid-state NMR for the quantitation of clarithromycin polymorph was evaluated.

Experimental

Materials Clarithromycin (form II) was supplied by Taisho Pharmaceutical Co., Ltd. Japan. Its recrystallization was carried out from the ethanol solution to obtain form I.

X-Ray Powder Diffraction (XRD) X-Ray powder diffraction was performed on a Rigaku Miniflex diffractometer (Japan). The measurement conditions were CuK α radiation, 30 kV voltage, 15 mA current with a scanning at 5° min⁻¹ over a 2 θ range between 5 and 40°. To investigate polymorphic changes induced by heating, XRD was carried out at elevated temperatures using a Philips X'Pert-MPD diffractometer (Netherlands) equipped with a Philips Japan TTK2-HC heat controller attachment model (Japan). Experimental conditions were as follows: CuK α radiation; voltage, 40 kV; current, 50 mA; scanning speed, 0.1° s⁻¹; scanning angle (2 θ), 3-40°; heating rate, 5°C min⁻¹.

Thermal Analysis Thermogravimetry-differential thermal analysis (TG-DTA) was carried out using MAC science TG-DTA 2000 (Japan) at a heating rate of 5°C min⁻¹ under a nitrogen gas flow.

Solid-State NMR Spectroscopy ¹³C-NMR spectra were measured on a JNM-LA400 NMR spectrometer (JEOL, Japan) operating at 100.4 MHz with a CP/MAS (cross-polarization/magic angle spinning) probe. The sample (ca. 190 mg) was contained in a cylindrical rotor made of ceramic materials, and spun at 6000 Hz. The contact time and the repetition time were fixed as 2 ms and 4.2 s, respectively. The spectral width and number of data points were 40 kHz and 16000, respectively; number of accumulations was 10000 to achieve an appropriate signal-to-noise ratio. Experimental conditions were as follows: temperature, 25.0°C; ¹H decoupling field amplitude,

50 kHz; rf field amplitude for cross polarization, 50 kHz.

Results and Discussion

Figure 1 shows DTA curves and XRD pattern of intact (form II) and recrystallized (form I) clarithromycins. Negligible weight loss was observed in both TG curves. Form II shows an endothermic peak at approximately 228°C owing to the melting of clarithromycin. An exothermic peak around 140°C was found in the DTA curve of form I, while no exothermic peak was found in that of form II. Characteristic diffraction peaks of forms II and I were observed at 2 θ =8.6° (closed circle) and 2 θ =4.5 and 6.5° (open circles), respectively.

Changes in the XRD pattern of form I during heating are shown in Fig. 2. A decrease in the intensity of characteristic X-ray diffraction peaks of form I was found with increase of heating temperature, and finally these peaks disappeared after heating at 140°C. When form I was heated at 130°C, characteristic X-ray diffraction peaks of form II appeared in the XRD pattern. The intensity of these peaks was gradually increased as the temperature was elevated. Since the diffraction pattern after heating at 140°C was closely similar to that of form II, the exothermic peak in Fig. 1(b) indicated a transformation from form I to stable form (form II). Therefore, the recrystallized clarithromycin (form I) was determined to be a metastable form.

For the two polymorphs of clarithromycin, notable differences were also observed in the solid-state ¹³C-NMR spectra (Fig. 3). Comparison of each spectrum in the high magnetic field showed the resonance of form II in the high magnetic field to be different from that of form I. Since the peaks in this field were more complicated than in the low magnetic field, it was difficult to distinguish the characteristic peak of each form. For this reason, we focused on the resonance around 175 ppm of each form. Figure 3(c) illustrates the expanded region of ¹³C-NMR spectra of equimolar mixture of form II and form I. These resonances observed around 175 ppm were assigned to the C1 carbonyl carbon of each form. Although the ¹³C chemical shift value of C1 carbonyl carbon of form II was close to that of form I, each peak of C1 carbon was well separated in the spectra of the mixtures of forms II and I at any mixing ratio. As shown in Fig. 3(c),

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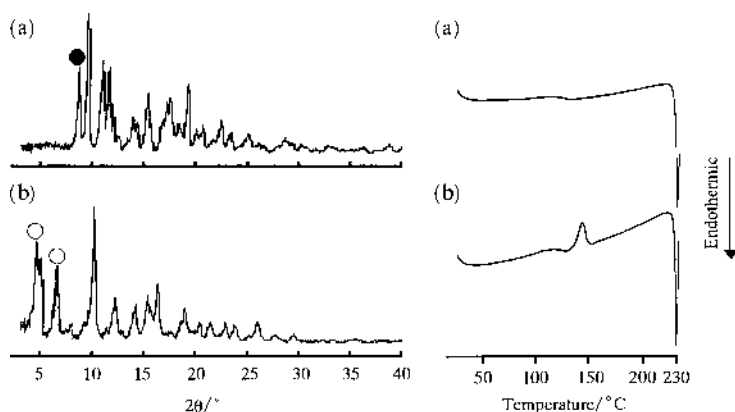


Fig. 1. Powder X-Ray Diffraction (XRD) Patterns and Differential Thermal Analysis Curves of Clarithromycin; (a) Form II, (b) Form I

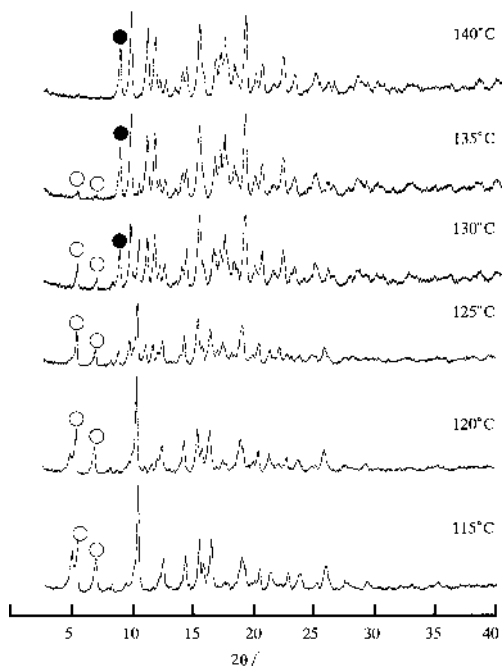


Fig. 2. Changes in Powder XRD Patterns of Form I by Heating from 115 to 140 °C

the intensity of C1 carbon resonance of form II was not identical to that of form I in the equimolar mixture of each form. Since the optimum parameters of the contact time and pulse decay time varied depending upon the sample,⁹ the optimal condition would also be different between two forms. In this experiment, contact time and pulse decay time were fixed at 2 ms and 4.2 s, respectively. Therefore, the intensity of C1 carbon resonance of form I was lower than that of form II in the equimolar simple mixture.

The polymorphic fraction using the ¹³C peak area was quantitatively analyzed. It was reported that the intensity measurements should be used for the integrated peak area rather than the peak height since different degrees of line broadening factor may be involved in the resonance of each polymorph.⁷ The peak areas at 176.2 ppm (form II) and at 175.2 ppm (form I) of the ¹³C-NMR of the mixtures were used for determination. Experimental and simulated ¹³C-NMR spectra for the C1 carbonyl carbons of each form are shown in Fig. 4. The values of S₂ and S₁ give the peak areas

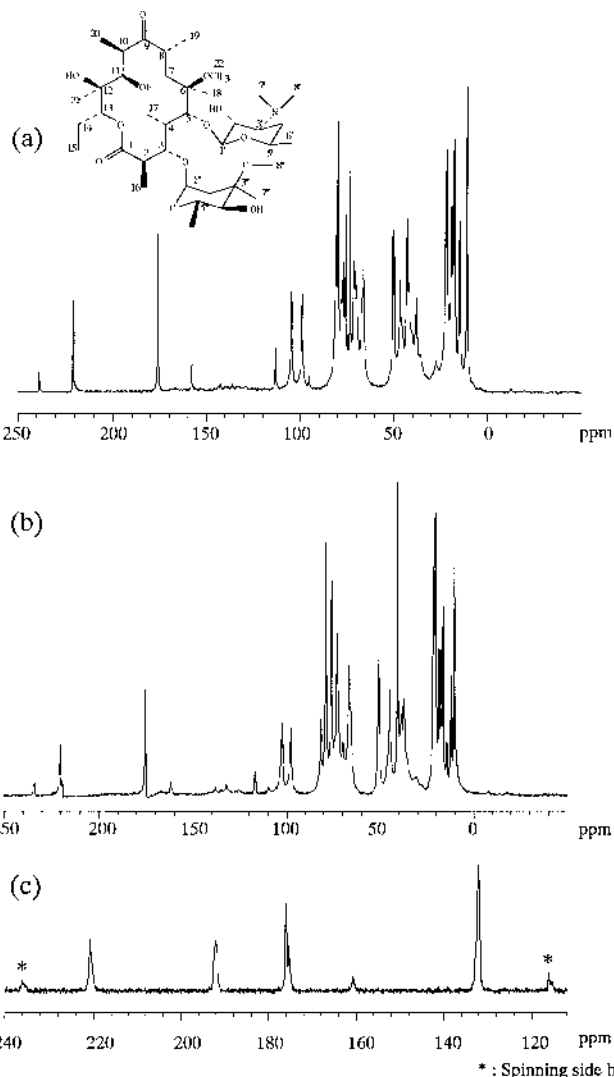


Fig. 3. ¹³C CP/MAS NMR Spectra of Clarithromycins; (a) Form II, (b) Form I, (c) Equimolar Mixture of Form II and Form I

of form II and form I, respectively. The peak areas of each form were estimated with computer-fitted curves using ALICE2 software (JEOL, Japan). Since the peak area of C1 carbon resonance of form I was lower than that of form II in the equimolar mixture, the peak area of form I should be normalized for quantitative analysis. The calibration factor α

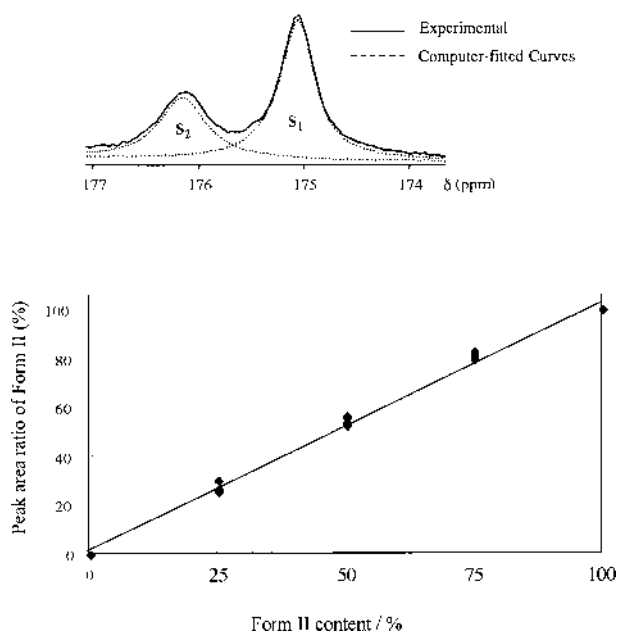


Fig. 4. Calibration Curve of Normalized Peak Area Ratio of Form II as a Function of Form II Content

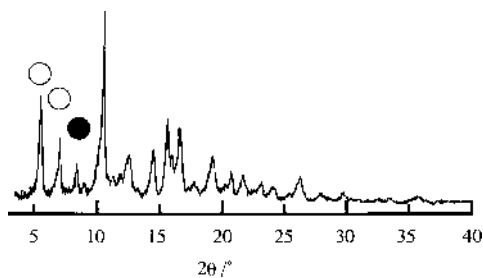


Fig. 5. Powder XRD Patterns of Form I after Heating at 140 °C for 4 min

was determined from the peak area ratio of form II to form I (S_2/S_1). The normalized peak area of form I (S_1') was calculated by multiplying of the peak area of S_2 by the calibration factor α ($S_1' = S_1 \times \alpha, \alpha = 1.12$). Simple mixtures containing different levels of form II with predetermined ratios were used as standards for quantitation. The measuring points of the mixtures were determined at five ratios (form II content

was 0, 25, 50, 75 and 100%) and were analyzed four times at each mixing ratio. Figure 4 shows the calibration plots of the form II peak area ratio in the binary mixtures as a function of form II content. A linear least squares analysis yielded a slope of 1.01, an intercept of 2.35%, and a coefficient determination R^2 of 0.993. Good linearity was obtained between the form II content and the normalized peak area ratio of that form. Figure 5 shows the powder XRD pattern of form I after heating at 130 °C for 4 min, which involves both characteristic X-ray diffraction peaks of each form. Using the calibration plot, the peak area of form II in this sample was estimated to be 35%, implying that 35% of form I was converted to form II by heating at 130 °C for 4 min. With regard to the solid-state ^{13}C -NMR spectra, the crystallinity affected the sharpness of the peaks in the spectrum.⁸⁾ Furthermore, the integrated intensity of the spinning side band of clarithromycin varied depending on the spinning condition. Although these various problems should be overcome for quantification, it is suggested that solid-state ^{13}C CP/MAS NMR is a plausible method for quantitative determination of polymorphism if an appropriate condition is selected.

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