

Thermal-Dependent Dehydration Process and Intramolecular Cyclization of Lisinopril Dihydrate in the Solid State

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The pathway of dehydration and intramolecular cyclization of lisinopril dihydrate in the solid state was investigated using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and a combination of thermal analyzer with Fourier transform infrared microspectroscopy (thermal FT-IR microscopic system). The results indicate that the dehydration from the solid-state lisinopril dihydrate had a two-step process from dihydrate to monohydrate at 76 °C and then from monohydrate to anhydrate at 99–101 °C, which could be clearly observed from the above three methods. Only the thermal FT-IR microscopic system could give vital information on diketopiperazine (DKP) formation *via* intramolecular cyclization in anhydrous lisinopril. A new peak at 1670 cm⁻¹ assigned to the carbonyl band of DKP formation was clearly evidenced. The water of reaction by-product was liberated at a temperature >157 °C and appeared on the IR spectra near 3200–3400 cm⁻¹. Moreover, the peak at 1574 cm⁻¹ assigned to carboxylate shifted to 1552 cm⁻¹ due to the DKP formation. The peak at 1670 cm⁻¹ related to the DKP formation changed slightly in intensity from 147 °C and significantly near 157 °C. DSC and TGA methods were poor for use in supplying information on DKP formation in lisinopril. The thermal FT-IR microscopic system is useful from the view point that it can quickly and directly show the solid-state stability of drug.

Key words lisinopril dihydrate; solid state; dehydration; intramolecular cyclization; thermal FT-IR microscopic system; DSC

Lisinopril as one of the long-acting angiotensin-converting enzyme (ACE) inhibitors is widely used as a clinical therapy for hypertension and heart failure, acting as a dihydrate in tablets by oral administration.^{1,2)} Since lisinopril is an *N*-carboxyalkyl lysyl-proline dipeptide analog, diketopiperazine (DKP) can easily form from the non-enzymatic intramolecular aminolysis in lisinopril, as true in other ACE inhibitors.^{3–6)} DKP that forms between two neighboring amino acids *via* intramolecular cyclization has also been recognized as a degradation pathway of peptides.^{7–10)} It may potentially appear as synthetic impurities or degradation products in the drugs or dosage forms causing a major stability problem in drug preparations. This intramolecular aminolysis reaction of lisinopril has been studied in solution,³⁾ but not in the solid state.

Our previous studies have successfully used a newly devel-

oped Fourier transform infrared (FT-IR) microspectroscopy combined with a thermal analyzer to simulate the accelerated stability test and simultaneously to study the thermal-related reaction *via* the structural changes in IR spectra of various samples.^{10–15)} This combined system is a simple, quick and powerful tool for investigation of the thermo-dependent characterization of samples. In this paper, the dehydration process of lisinopril dihydrate and the pathway of DKP formation of the solid-state lisinopril were studied using this thermal FT-IR microscopic system. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were used for comparison.

Experimental

Materials Lisinopril dihydrate of pharmaceutical grade was purchased from Chemical Works Gedeon Richter Ltd., Hungary and was kindly donated by China Chemical & Pharmaceutical Co. Ltd, Taipei, R.O.C. KBr was

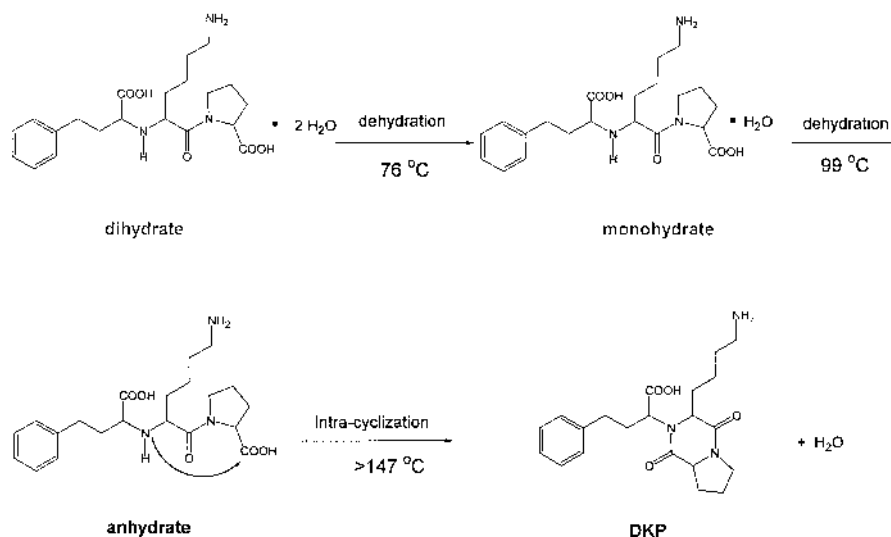


Chart 1. Pathway of the Dehydration Process and Intramolecular Cyclization of Solid-State Lisinopril Dihydrate

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of analytical reagent grade, purchased from Nakalai Tesque, Kyoto, Japan.

Thermal Analysis The crystals of lisinopril dihydrate with or without preheating were examined by DSC (DSC-910, TA Instru. Inc., New Castle, DE, U.S.A.) at a heating rate of 3 °C/min with an open pan system in a stream of N₂ gas. TGA (TGA-951, TA Instru. Inc., New Castle, DE, U.S.A.) was also performed at the same heating rate to determine the weight loss of sample.

Thermal FT-IR Microspectroscopic Study^{10–15} A small amount of lisinopril dihydrate was sealed within two pieces of KBr pellets by hydraulic press. This compressed KBr disc was directly put on a DSC microscopy cell (FP 84, Mettler, Greifensee, Switzerland) and the cell was then placed on the stage of the microscope within the FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Tokyo, Japan) by a mercury-cadmium-telluride (MCT) detector. The system was operated in the transmission mode. The position and focus of sample were adjusted by the microscope. Temperature of DSC microscopy cell was monitored with a central processor (FT80HT, Mettler). The heating rate of the DSC assembly was controlled at 3 °C/min in ambient condition. During the experiment, the sample disc was first equilibrated to the starting temperature (25 °C) for about 3 min and then heated from 25 to 200 °C. The thermal-responsive IR spectra were recorded while the sample disc was heated on the DSC microscope stage.

Results and Discussion

The DSC thermograms and TGA curve of lisinopril dihydrate are depicted in Fig. 1. Three main endothermic peaks at 76, 99 and 167 °C were observed from the DSC thermograms of the solid-state sample of lisinopril dihydrate (Fig. 1, a). In the same temperature range, two obvious steps of weight loss before 120 °C were seen from the TGA curve. The first loss in the curve corresponding to the endothermic peak at 76 °C was about 4.0%, and the second loss corresponding to the endothermic peak at 99 °C was also about 4.0%. About 1% of weight loss found at the initial stage was due to evaporation of the adsorbed water. The 4.0% weight loss was equal to almost 4.07% of water loss from lisinopril dihydrate and to 4.25% of water loss from lisinopril monohydrate (molecular weight of lisinopril dihydrate: 441.5). This suggests that the endothermic peaks at 76 and 99 °C might be the result of a two-step dehydration process from the solid sample. The endothermic peak at 167 °C might have to do with the melting of the sample, which will be discussed in the following section. To confirm the two-step dehydration, the solid-state sample of lisinopril dihydrate was pre-heated to 80 or 100 °C, respectively, and then again determined by DSC. It is evident that the endothermic peak at 76 °C disappeared from the DSC curve of the 80 °C-preheated sample, but the peaks at 95 and 167 °C remained (Fig. 1, b). Both endothermic peaks at 76 and 99 °C disappeared from the DSC curve of the 100 °C-preheated sample, but the peak at 167 °C remained (Fig. 1, c). This strongly indicates that dehydration from the solid-state lisinopril dihydrate occurred in a two-step process from dihydrate to monohydrate and then from monohydrate to anhydride.

Figure 2 shows the three-dimensional plots of FT-IR spectra of lisinopril dihydrate as a function of temperature between 3700–2500 and 1800–1200 cm⁻¹ wavenumbers. The peak at 3554 cm⁻¹ is assigned to the stretching vibrations of an O–H band of water. The peaks at 3340 and 3296 cm⁻¹ are due to the asymmetric and symmetric N–H band of primary amine with hydrogen bonding. The peak at 3099 cm⁻¹ is due to the aromatic C–H stretching, and the peaks at 2962 and 2925 cm⁻¹ are assigned to the asymmetric C–H stretching vibrations, respectively. The peak at 1655 cm⁻¹ is attributed to the carbonyl stretching of tertiary amide and/or scissoring

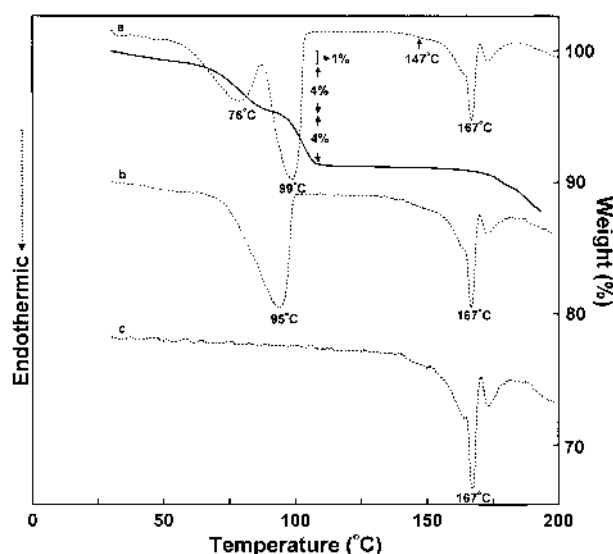


Fig. 1. DSC Thermograms (Dotted Line) and TGA Curve (Solid Line) of Lisinopril Dihydrate

(a) Sample without preheating, (b) 80 °C-preheated sample, (c) 100 °C-preheated sample.

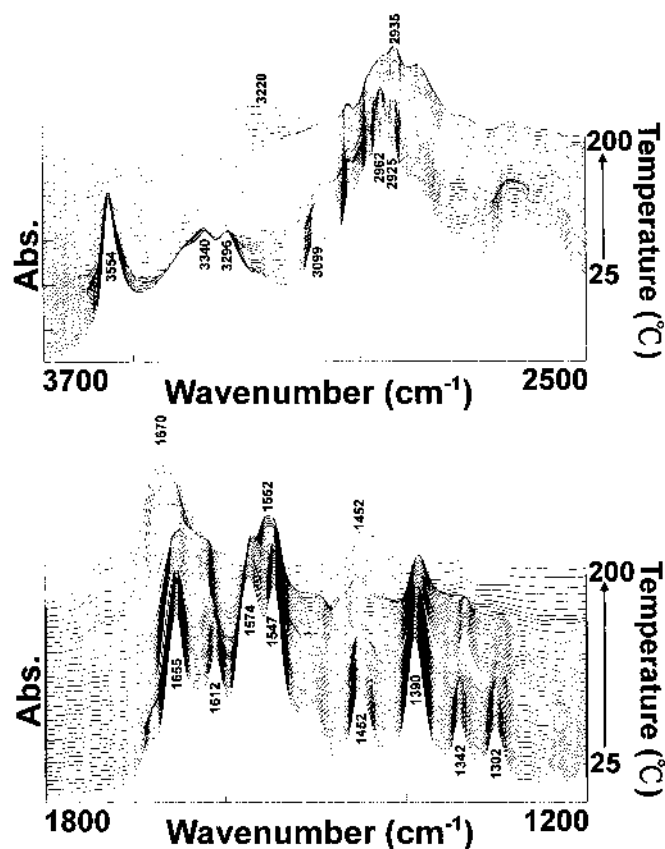


Fig. 2. Three-Dimensional Plots of FT-IR Spectra of Lisinopril Dihydrate as a Function of Temperature

NH₂; that at 1612 cm⁻¹ is due to the aromatic ring mode, and those at 1574 and 1547 cm⁻¹ correspond to the asymmetric carboxylate and/or ring mode of the aromatic group; the peak at 1452 cm⁻¹ indicates the CH₂ scissoring mode and the peak at 1390 cm⁻¹ is due to the symmetric carboxylate.¹⁶ With the increase of temperature, the IR spectral contour and several frequencies corresponding to the water (3554 cm⁻¹), hydro-

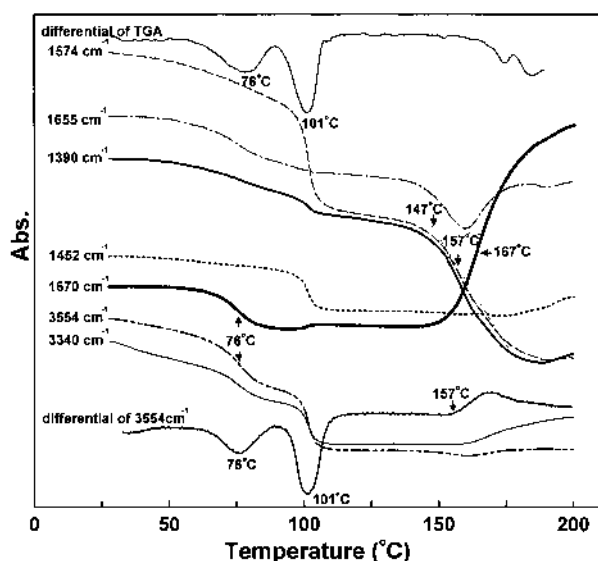


Fig. 3. Temperature-Dependent Changes in Peak Intensity of the Selected IR Bands of Lisinopril Dihydrate

gen bonding of primary amine (3340 , 3296 cm^{-1}) and carboxylate (1574 , 1390 cm^{-1}) significantly changed. Moreover, a new peak at 1670 cm^{-1} assigned to the carbonyl band of DKP formation due to intramolecular cyclization was clearly evidenced.^{10,16,17} The conformational changes result in the disappearance of hydrogen-bonding bands and the formation of DKP in solid-state lisinopril dihydrate. The water of the reaction by-product was liberated after the temperature reached 157°C and appeared on the broad IR spectra near about $3200\text{--}3400\text{ cm}^{-1}$. The persistence of the IR spectra of water ranging from $3200\text{--}3400\text{ cm}^{-1}$ was still observed even at higher temperature and might due to the by-product "water" being tightly sealed within KBr discs. In addition, the interaction of water with KBr might induce the peak at 3500 cm^{-1} (assigned to free water) to shift to a lower wave-number ($3200\text{--}3400\text{ cm}^{-1}$). Due to the DKP formation the peak at 1574 cm^{-1} assigned to carboxylate shifted to 1552 cm^{-1} .

To verify the dehydration process and DKP formation from lisinopril dihydrate, the changes in IR peak intensity of several specific bands with temperature are displayed in Fig. 3. Obviously, the peak intensity of these specific bands of lisinopril dihydrate decreased gradually with temperature and were decreased apparently, especially near $76\text{--}101^\circ\text{C}$. The bands at 3554 (νOH) and 3340 (νNH) cm^{-1} were observed with two-step change, as confirmed by the differential curves of TGA and the 3340 cm^{-1} band. Factors such as crystal packing, nucleation and hydrogen bonding are assumed to influence solid-state desolvation reaction.^{18,19} The overall dehydration process might consist of two simple processes: the breaking of hydrogen bonding and the rapid escape of water from hydrate. The former is more related with IR spectral change, while the latter is easily obtained by TGA. In the present study, dehydration was found to undergo a two-step process at the same temperature, one with a differential curve of the 3340 cm^{-1} band due to the breaking of hydrogen bonding, and the other with the differential TGA curve due to the escape of water from hydrate. The breaking of hydrogen bonding was a rate-determining step. Other peaks at 1574 ,

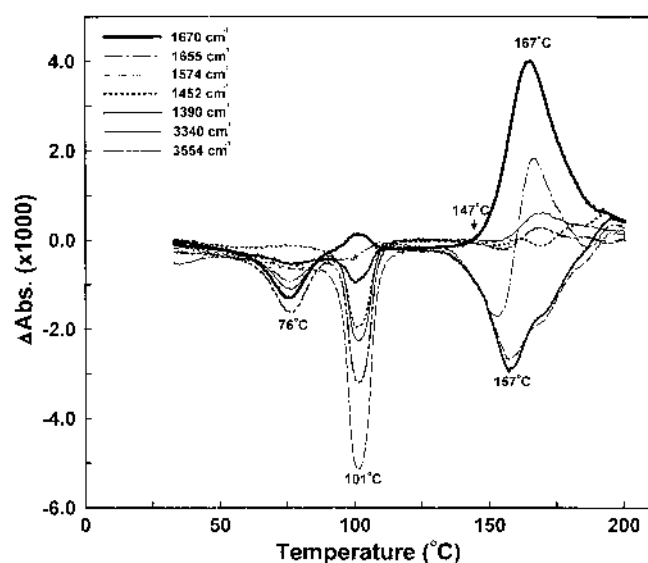


Fig. 4. Differential Curves of Peak Intensity of the Selected IR Bands of Lisinopril Dihydrate

1390 and 1542 cm^{-1} changed in peak intensity at 101°C , and the peak at 1655 cm^{-1} altered more pronouncedly from 76°C . Although the peak at 1670 cm^{-1} also showed a slight change within $76\text{--}101^\circ\text{C}$, the overlapping of 1655 cm^{-1} might be responsible for this.

The dehydration process seemed to induce the structural transformation of lisinopril dihydrate to change the IR peak intensity of different functional bands with temperature. The peaks at 1655 , 1574 and 1390 cm^{-1} assigned to the carboxylate groups reduced in intensity gradually from above 147°C and markedly at 157°C . In the meantime, the appearance of 1670 cm^{-1} also slightly changed in peak intensity from 157°C but was significantly altered near 167°C . Moreover, the peak intensity at 3340 cm^{-1} (N-H band of primary amine), also assigned to the stretching OH vibration of water increased again from 157°C . This strongly implies that DKP and water can be produced by the thermal-dependent intramolecular cyclization in anhydrous lisinopril between $147\text{--}157^\circ\text{C}$. The changes of FT-IR spectra give more evidence of the DKP formation in lisinopril than do those from DSC thermogram. Three main processes corresponding to dehydration were also observed in the differential IR bands: from dihydrate to monohydrate at 76°C , from monohydrate to anhydrate at 101°C , and DKP formation *via* intramolecular cyclization at $>147^\circ\text{C}$ (Fig. 4). The process of dehydration and DKP formation of solid-state lisinopril dihydrate is postulated in Chart 1.

To verify that the DKP formation *via* intramolecular cyclization was a solid state reaction, an isothermal study of solid-state lisinopril dihydrate was made at 135°C before the fusion point of 167°C . Figure 5 shows the three-dimensional plots of IR spectra of lisinopril dihydrate between 1800 and 1200 cm^{-1} , with respect to isothermal time. Only a slight shift in IR spectra for the sample was found due to the difference in temperature (135 , 25°C). With the increase of isothermal time at 135°C , however, the peaks at 1612 and 1651 cm^{-1} gradually disappeared and were replaced by a broad spectrum near 1651 cm^{-1} . If the sample was directly changed to a non-isothermal study from 135°C (solid state)

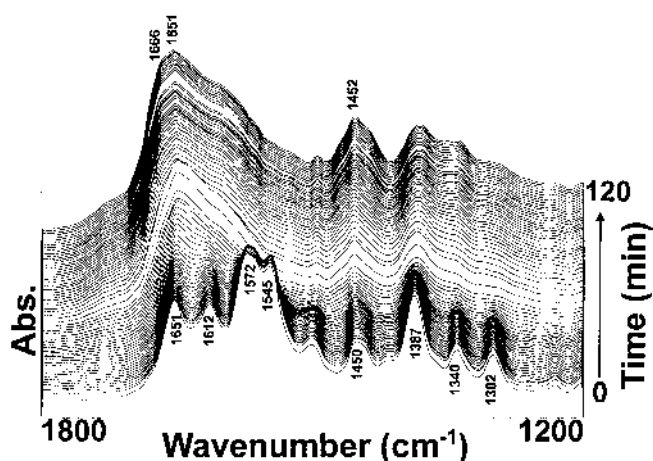


Fig. 5. Three-Dimensional Plots of FT-IR Spectra of Lisinopril Dihydrate between 1800 and 1200 cm^{-1} with Respect to the Isothermal Time at 135 °C

to 200 °C (liquid state), the IR peak at 1651 cm^{-1} shifted to 1670 cm^{-1} . This broad spectrum found at 1651 cm^{-1} might be assigned to the solid-state DKP formation through the isothermal study at 135 °C, implying that the DKP formation occurred in the solid-state lisinopril. Thus, it should be noted that the fusion peak at 167 °C in the DSC curve might be attributed to the melting of DKP rather than to lisinopril.

In conclusion, the thermal-dependent dehydration process and intramolecular cyclization of lisinopril dihydrate in the solid state were effectively studied using a novel FT-IR microscope equipped with differential scanning

calorimetry. Two-step dehydration was clearly evidenced from the changes in FT-IR spectra. Moreover, the solid-state DKP formation in lisinopril *via* intramolecular cyclization was also quickly and directly investigated by this thermal FT-IR microscopic system.

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