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Note

Intramolecular cyclization of diketopiperazine formation in solid-state enalapril maleate studied by thermal FT-IR microscopic system

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Abstract

The pathway of diketopiperazine (DKP) formation of solid-state enalapril maleate has been studied by using a novel Fourier transform infrared microspectroscope equipped with a thermal analyzer (thermal FT-IR microscopic system). The thermogram of the conventional differential scanning calorimetry (DSC) method was also compared. The results show new evidence of IR peaks at 3250 cm^{-1} (the broad O-H stretching mode of water), and at 1738 and 1672 cm^{-1} (the carbonyl band of DKP), indicating DKP formation in enalapril maleate via intramolecular cyclization. Moreover, the disappearance of IR peaks from enalapril maleate at 3215 cm^{-1} (the secondary amine), 1728 cm^{-1} (the carbonyl group of carboxylic acid), and 1649 cm^{-1} (the carbonyl stretching of tertiary amide) also confirmed the DKP formation. The thermal FT-IR microscopic system clearly evidenced that the DKP formation in enalapril maleate started from 129 °C, and reached a maximum at 137 °C. This result was also confirmed by the conventional DSC thermogram of the compressed mixture of KBr powder and enalapril maleate, in which an endothermic peak at 144 °C with an extrapolated onset temperature at 137 °C was observed. This strongly suggests that the thermal FT-IR microscopic system was able to qualitatively detect the formation of DKP derivatives in solid-state enalapril maleate via intramolecular cyclization.

Keywords: Enalapril maleate; Diketopiperazine; Thermal Fourier transform infrared microscopic system; Solid-state intramolecular cyclization; Differential scanning calorimetry; Thermogravimetric analysis

1. Introduction

Enalapril is an angiotensin converting enzyme (ACE) inhibitor of *N*-carboxyalkyl dipeptide developed for treatment of hypertension and congestive heart failure [1]. Like other ACE inhibitors, enalapril is liable to degrade to the enalaprilat and enalapril diketopiperazine (DKP) in aqueous solution [2–5]. The formation of DKP between two neighboring amino acids via intramolecular cyclization has also been recognized as a degradation pathway [6–9]. A detailed stereochemical analysis of DKP derivatives of enalapril has been made using NMR spectroscopy [5]. The DKP derivatives might potentially cause synthetic impurities in the drug formulations, resulting in instability of solid dosage forms [10]. There are many studies on the stability of enalapril in solution [2–5], however, no detailed investigation of the degradation pathway of enalapril maleate in the solid state has been made.

In our laboratory, a novel and powerful Fourier transform infrared (FT-IR) microspectroscope equipped with a thermal analyzer has been used to study the structural changes associated with thermal response for various samples such as drugs, skin, polymer, and proteins [11–15]. This innovative thermal FT-IR microscopic system which uses the transmission or reflectance method is a simple, quick and powerful tool for the simultaneous investigation of thermal response and infrared spectra of samples. The purpose of this study is to quickly and qualitatively investigate the pathway of DKP formation in solid-state enalapril maleate using this thermal FT-IR microscopic system.

2. Materials and methods

2.1. Materials

Enalapril maleate used in this study was of pharmaceutical grade, was purchased from Chem. Works Gedeon Richter Ltd., Hungary and was kindly donated by China Chem. & Pharm. Co. Ltd, Taipei, ROC. The KBr powder and KBr crystal for pellet were obtained from Nakalai

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Tesque (Kyoto, Japan) and Jasco Parts Center (Jasco Co., Tokyo, Japan), respectively.

2.2. Thermal analysis of enalapril maleate

The powdered sample of enalapril maleate was examined by conventional differential scanning calorimetry (DSC) (DSC-910, TA Instruments Inc., USA) at a heating rate of 3 °C/min with an open pan system in a stream of N_2 gas. The DSC cell was calibrated with indium. The mixtures (weight ratio 3:1) of KBr powder and enalapril maleate with or without compression were also determined by DSC. The compression was performed by a hydraulic press (Riken Seiki Co., Tokyo, Japan) under 200 kg/cm^2 for 15 s. Thermogravimetric analysis (TGA) (TGA-951, Dupont, USA) was also performed at the same heating rate.

2.3. Thermal FT-IR microscopic study

The powdered sample of enalapril maleate was sealed within two pieces of KBr pellet by hydraulic press (200 kg/cm^2, 15 s). The compressed KBr disc was put directly on a micro hot stage (DSC microscopy cell, FP 84, Mettler, Switzerland). The DSC microscopy cell was then placed on the stage of the microscope in the FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Japan) with a MCT detector. The system was operated in the transmission mode. The position of the sample and the focus were adjusted using the microscope. The IR beam was imaged onto the sample with a 16× Cassegrainian objective. The desired sample size for determination was selected and defined by means of an aperture through optical system (ATOS). The temperature of the DSC microscopy cell was monitored with a central processor (FP 80HT, Mettler, Switzerland). The heating rate of the DSC assembly was controlled at 3 °C/min under ambient conditions. The sample disc was previously equilibrated to the starting temperature (25 °C) for about 3 min and then heated from 25 to 160 °C. The DSC cell was calibrated with indium, and the polystyrene film was used as a wavenumber calibrator of the FT-IR microscopic spectrometer. The thermal-responsive IR spectra were recorded with respect to the temperature [11–15]. A small amount of pure enalapril maleate was also put on a drug-free KBr disc without compression and then determined by this thermal FT-IR microscopic system.

3. Results and discussion

Fig. 1 shows the three-dimensional plots of FT-IR spectra of enalapril maleate sealed within a KBr pellet as a function of temperature between 3500–2700 and 1850–1150 cm^{-1} wavenumbers. It is evident that with the increase of temperature, the frequency of each IR peak for enalapril maleate remains almost constant up to 129 °C and then dramatically changes. The peaks at 3215 and 3025 cm^{-1} are assigned to the stretching vibrations of N-H and C-H bands of aromatic ring. The peaks at 2979 and 2929 cm^{-1} are due to the asymmetric CH_3 and CH_2 stretching vibrations, respectively. The peaks at 1751 and 1728 cm^{-1} are attributed to the carbonyl stretching of ester and carboxylic acid; the peak at 1649 cm^{-1} corresponds to the carbonyl stretching of tertiary amide; peaks at 1574 and 1493 cm^{-1} are assigned to the carboxylate of maleate and/or ring mode of benzene; the peak at 1454 cm^{-1} corresponds to CH_2 scissoring; the peak at 1379 cm^{-1} is due to C-H bending; peaks at 1230 and 1192 cm^{-1} correspond to the C-C-O stretching bands of acetate and ester, respectively. A number of IR peaks disappeared, but several new peaks at 3250, 2985, 1738 and 1672 cm^{-1} were observed. These new peaks except 3250 cm^{-1} were also found in the IR spectra of DKP derivatives (2981, 1739 and 1672 cm^{-1}), as reported by Demeter et al. [5]. The broad spectra at 3250 cm^{-1} might be attributed to the O-H stretching mode of water, the peak at 2985 cm^{-1} was due to the asymmetric CH_3 stretching vibration, and the peaks at 1738 and 1672 cm^{-1} were assigned to the carbonyl bands of ester and DKP groups, respectively [16]. The appearance of IR peaks for water and for DKP derivatives implies that the enalapril maleate forms enalapril DKP via intramolecular cyclization during the heating process. The postulated pathway of intramolecular cyclization in enalapril is shown in Scheme 1.

In order to verify the formation of DKP derivatives via intramolecular cyclization in enalapril maleate, the temperature-dependent changes in peak intensity of several of the above IR bands for enalapril maleate sealed within a KBr pellet and for DKP derivatives formed are illustrated in Fig. 2. The conventional DSC curve of the compressed mixture of KBr powder and enalapril maleate is also shown for comparison. The peak intensity of the IR bands for enalapril maleate (3215, 1751 and 1649 cm^{-1}) remained almost constant as the temperature was increased to 129 °C and then dramatically decreased from 137 °C. At the same time, the peaks at 1738 and 1672 cm^{-1}, which were assigned to the carbonyl group of DKP derivatives, also appeared gradually from 129 °C and strengthened at 137 °C. Moreover, the peak at 3215 cm^{-1}, due to the secondary amine, disappeared but the peak at 3250 cm^{-1} corresponding to the O-H stretching mode of water was noted, strongly indicating the formation of DKP derivatives in enalapril maleate sealed within a KBr pellet via intramolecular cyclization from 129 °C but the maximum reaction accelerated from 137 °C. This was consistent with the conventional DSC curve of the compressed mixture of KBr powder and enalapril maleate (Fig. 2, DSC), in which the onset and extrapolated onset temperatures were observed at 129 and 137 °C, respectively.

Fig. 3 shows the conventional DSC thermograms of different samples of enalapril maleate. An endothermic peak at 151 °C with an extrapolated onset temperature at 147 °C was observed for pure enalapril maleate, suggesting that the fusion of pure drug occurred between 147 and 151 °C (Fig. 3, DSC1). As the temperature was beyond 151 °C,

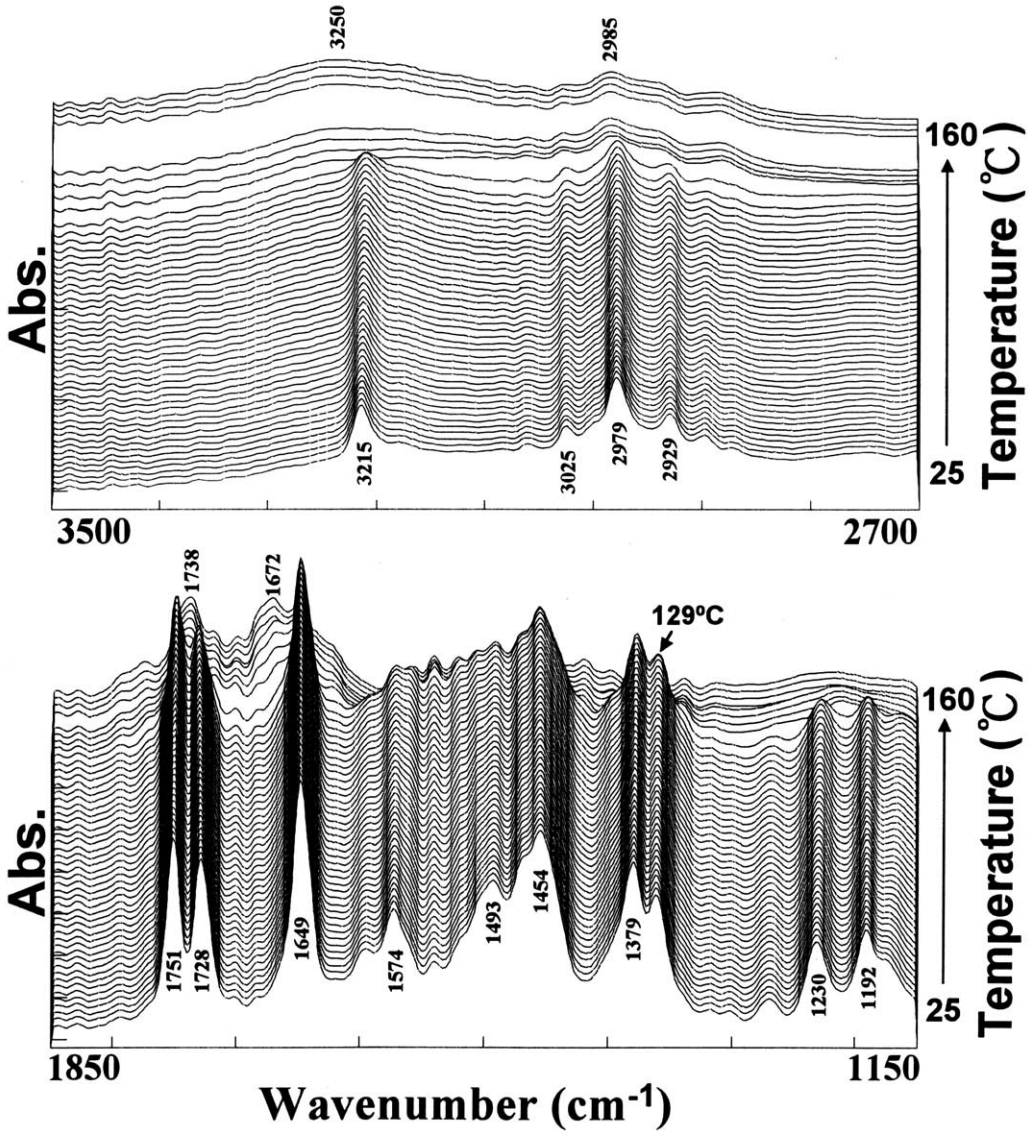
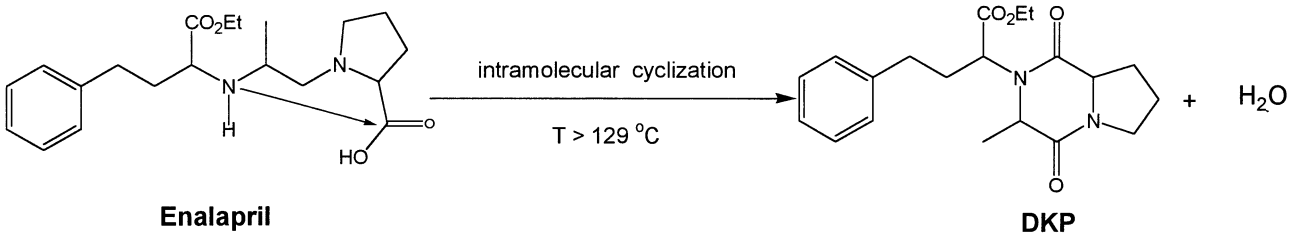


Fig. 1. Three-dimensional plots of FT-IR spectra of enalapril maleate sealed within a KBr pellet as a function of temperature between 3500–2700 and 1850–900 cm^{-1}.

another endothermic shoulder at 163 °C was evidenced on the conventional DSC curve. This endothermic shoulder peak might be due to the complex reaction [5], as seen from the changes in the TGA curve. Moreover, the weight loss of pure enalapril maleate between 147 and 220 °C was determined to be about 24.9 ± 2.4% ($n = 3$) from the TGA curve. This value was larger than that of the weight loss of water, implying that the complex reaction including DKP formation was also occurring. The TGA data also confirmed the DKP formation from 147 °C in pure enalapril.



Scheme 1. The pathway of intramolecular cyclization of enalapril maleate determined by thermal FT-IR microspectroscopy.

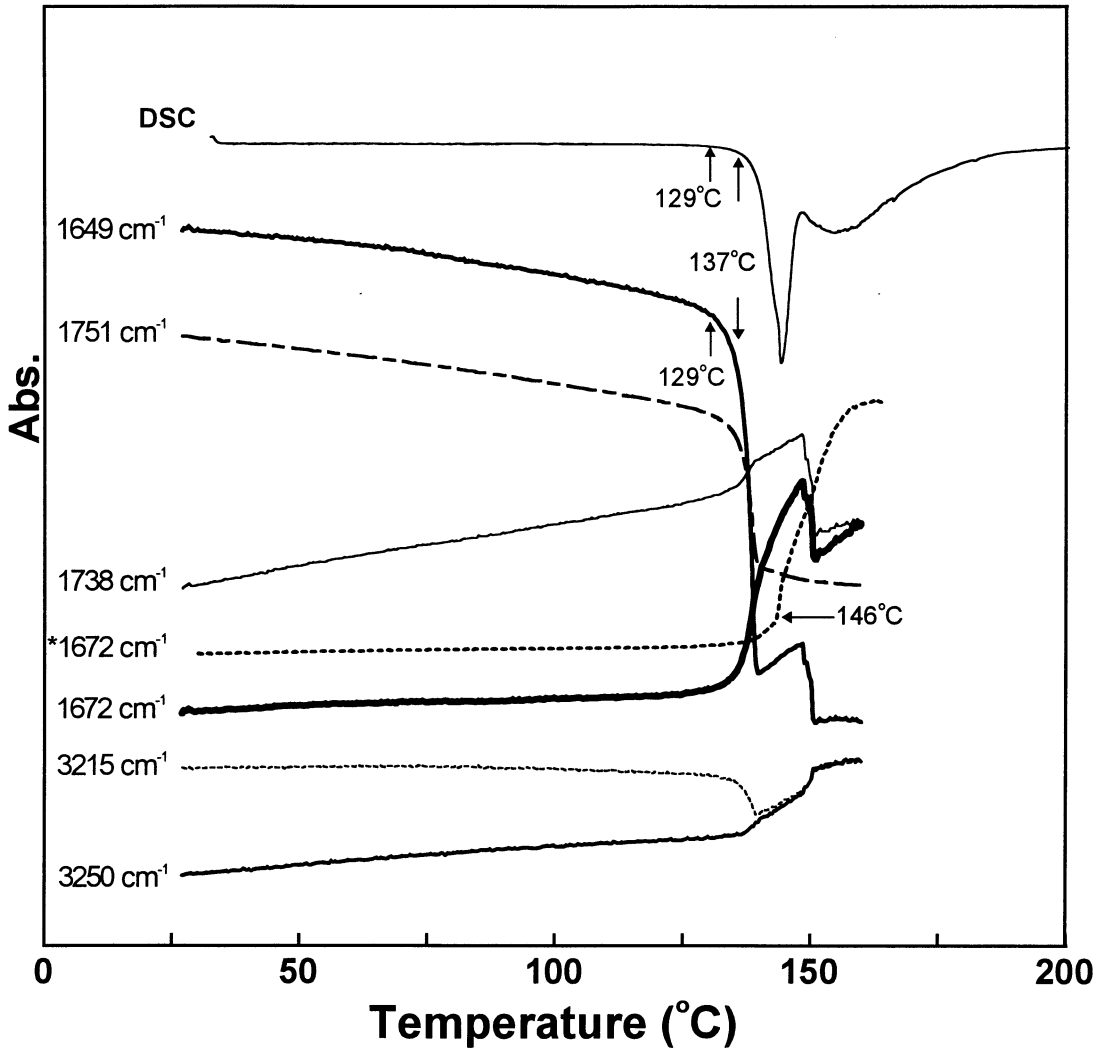


Fig. 2. The conventional DSC and temperature-dependent IR spectral changes in the peak intensity of selected bands of enalapril maleate sealed within a KBr pellet. * indicates the result of enalapril maleate powder put on the KBr disc without compression.

When KBr powder was mixed with pure enalapril maleate (weight ratio 3:1), the DSC endothermic peak of this mixture was observed at 148 °C with an extrapolated onset temperature at 140 °C (Fig. 3, DSC2). The endothermic peak at 148 °C was near to that of the pure drug at 151 °C, suggesting there was no interaction between KBr and enalapril maleate. Once the above mixture was compressed, however, the DSC endothermic peak of the compressed mixture shifted from 148 °C to 144 °C and had an extrapolated onset temperature at 137 °C (Fig. 3, DSC3). This indicates that the enalapril maleate compressed with KBr powder markedly changed its thermal sensitivity. The tight compaction after compression might shorten the distance of particles and easily induce the thermal conductivity for molecular interaction, leading to a faster fusion for the compressed sample than for the uncompressed or pure sample. From the above results of FT-IR and DSC, it is interesting to note that the compressed mixture determined by both thermal FT-IR and DSC methods exhibited the same temperature range from 129 to 137 °C for DKP formation. When putting a small amount of pure enalapril maleate on a drug-free KBr disc without compression followed by determination by this thermal FT-IR microscopic system, the representative IR peak intensity at 1672 cm^{-1}, assigned to carbonyl bands of DKP, showed a marked change from 146 °C (Fig. 2, *1672 cm^{-1}). The temperature at 146 °C was near to the onset temperature at 147 °C in the DSC curve for pure drug (Fig. 3, DSC1). This suggests that the compression effect plays an important role.

To verify that the DKP formation via intramolecular cyclization was a solid-state reaction, an isothermal study of solid-state enalapril maleate was made at 120 °C before the fusion point of 151 °C. Fig. 4 shows the three-dimensional plots of IR spectra of enalapril maleate between 1800 and 1300 cm^{-1}, with respect to isothermal time. With the increase of isothermal time at 120 °C, the peaks at 1751, 1728 and 1649 cm^{-1} gradually disappeared from 22 min but new peaks at 1738 and 1672 cm^{-1} appeared. The peaks at

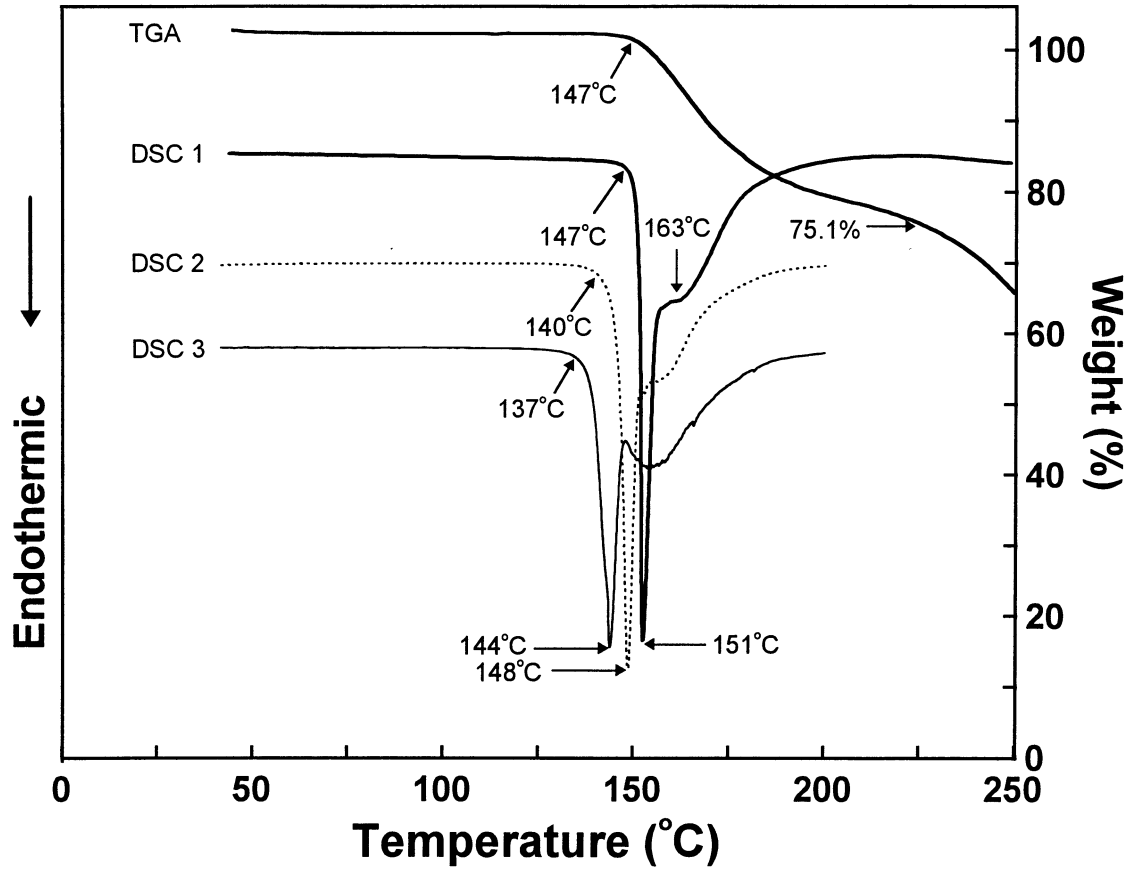


Fig. 3. The conventional DSC and TGA curves of different samples of enalapril maleate. DSC-1 & TGA, pure enalapril maleate; DSC-2, the mixture of KBr powder and enalapril maleate; DSC-3, the compressed mixture of KBr powder and enalapril maleate.

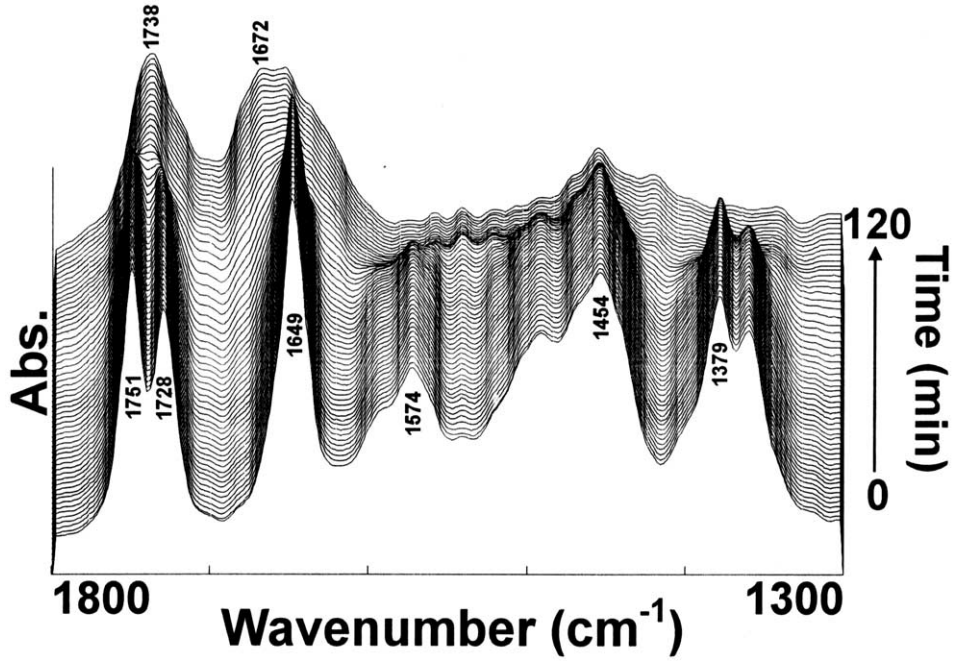


Fig. 4. Three-dimensional plots of FT-IR spectra of enalapril maleate sealed within a KBr pellet with respect to the isothermal time at 120 °C.

1751, 1728 and 1649 cm^{-1} are attributed to the carbonyl stretching of ester and carboxylic acid, and the carbonyl stretching of tertiary amide of enalapril maleate. The peaks at 1738 and 1672 cm^{-1} are assigned to the carbonyl group of DKP derivatives, suggesting solid-state DKP formation through the isothermal study at 120 °C. This also confirmed that the DKP formation occurred in the solid-state enalapril maleate.

In conclusion, the thermal FT-IR microscopic system was easy and useful for qualitatively detecting the DKP formation in solid-state enalapril maleate via intramolecular cyclization.

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