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High resolution spectroscopic evidence and solution calorimetry studies on the polymorphs of enalapril maleate

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Summary

Enalapril maleate, a potent angiotensin converting enzyme inhibitor, exists as polymorphs, Form I and Form II. X-Ray powder diffraction measurements have shown slightly different patterns. Differential scanning calorimetric thermograms failed to show any significant differences during melting. High resolution spectroscopic techniques, including solid state carbon-13 NMR, Fourier-transform IR and Raman, detect differences between Form I and Form II. Heats of solution data obtained also indicate measurable energy differences. It was concluded that these two polymorphic forms of enalapril maleate are energetically very similar. Virtual equivalence of in vitro dissolution rate was obtained from formulations of enalapril maleate made from either Form I, or Form II, or mixtures.

Introduction

Polymorphism is the rule rather than the exception for most drug entities. It has also been well documented that polymorphs exhibit different physical and chemical properties (Haleblian et al., 1969; Haleblian, 1975; Byrn, 1982). For pharmaceutical dosage forms, it is critical to determine whether or not these physical or chemical differences among polymorphs may impact on the performance of the dosage form.

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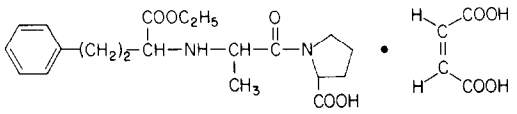
Physical-chemical differences between polymorphs are usually detected by conventional techniques, e.g. solubility, melting point and differential scanning calorimetry (DSC). While other techniques measure energy difference between different polymorphs, X-ray powder diffraction (XRPD) patterns reflect the atomic position in crystals and thus detect polymorphism directly attributable to real differences in crystal structure. Differences in XRPD patterns, therefore, provide good evidence for polymorphism. It is one of the most widely used techniques to identify polymorphs of pharmaceuticals (Trivedi et al., 1959; Biles, 1963; Mesley et al., 1968; Chapman et al., 1968; Mesley, 1971).

The presence of polymorphs for enalapril maleate (I) was first suggested by slight differences

in XRPD patterns. Data for solubility and heat of fusion, however, showed little differences among



Enalapril Maleate (I)

the forms. No correlation between XRPD patterns, heat of fusion, and solubility data could be established. We therefore undertook this study to pursue these observations. High resolution spectroscopic techniques and data on heats of solution were applied in an effort to confirm or deny the existence of polymorphs. In addition, solution calorimetry was used to establish the thermodynamic relationship between the polymorphs that were confirmed to exist.

Materials and Methods

Enalapril maleate, (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline maleate (Merck), was at least 98% pure. The polymorph designated as Form I was prepared by crystallization from ethyl acetate in the presence of methanol (3.5% by weight of methanol to ethyl acetate mother liquor) at room temperature (20–30°C). Water slurry of Form I at 5°C generates the polymorph designated as Form II.

Differential scanning calorimetry measurements were made on a Perkin Elmer 1-B using crimp sealed cups under a stream of nitrogen. A heating rate of 2.5°/min was employed. Data were analyzed on a Minc 11/23 minicomputer system.

Solubility analyses were done in acetonitrile/isopropyl acetate (1/1, by volume) after overnight agitation in a water bath at 25°C.

Dissolution studies were carried out in water with USP paddles at 50 rpm at 37°C. Samples for testing were removed after 30 min agitation. Analyses were performed on a reversed phase, 10 μm Lichrosorb RP-8, 200 × 4.6 mm i.d. analytical column (Hewlett Packard). Mobile phase consisted of a mixture of phosphate buffer (0.02 M, pH 4.0), 70%, and acetonitrile, 30%. Flow rate was set at 2.0 ml/min, column temperature at 80°C and detection wavelength at 215 nm. Samples of capsules and tablets were supplied by the Pharmaceutical Development Group and were representative of material used for ongoing clinical studies.

For XRPD a powder sample of enalapril maleate was gently pressed to a flat surface in a glass slide and the scintillation response measured (ordinate) versus 2θ value (abscissa) over the range of 3° to 40° 2θ. A strip-chart recorder was used on the Philips Electronics X-ray diffractometer (CuK_α radiation).

Solid State Carbon-13 NMR spectra were obtained using a Varian XL100 with a custom built magic angle spinning probe. An in-house built computer control network was used.

Infrared spectra were taken as potassium bromide pellets with a Nicolet 7199 Fourier-transform IR spectrometer. The resolution was 1 cm^{-1}. A global source, potassium bromide optics, and a DTGS detector were used.

Raman spectra were taken from the smoothed surface of the solid gently pressed into a solid sample holder. The sample was irradiated with 250–500 mW of 5145A radiation from a Spectra Physics 171-19 argon ion laser. A 90° scattering geometry was used. The scattered radiation was dispersed with a Jobin-Yvon Ramanor HG2S monochromator and detected with an S-20 (C31034) photomultiplier with associated photon-counting electronics (Princeton Applied Research Model 1140A). The system was interfaced to a Nicolet 1180 computer. The resolution was approximately 3 cm^{-1} in the region of interest.

Heats of solution were measured in a TRONAC-450 Solution Calorimeter. Samples (40–80 mg) were weighed into glass bulbs which were sealed and installed on the stirrer-bulb breaking device. The sample, stirrer, thermistor and calibration heater were immersed in the calorimetric vessel, a silvered dewar flask containing 40 ml of solvent. The entire assembly was lowered into a constant temperature bath held at 25.0°C.

After a stable baseline was established on the temperature vs time recorder, the system was calibrated with the calibration heater. Voltage and current were monitored with a digital voltimeter and the elapsed time measured with a digital timer.

The run was initiated by breaking the glass bulb, thus allowing the sample to come into contact with the solvents.

Periodically the accuracy of the calorimetric system was verified by measuring ΔH for the protonation of THAM (Tris-hydroxymethylaminomethane) (exothermic) and heat of solution of potassium chloride in water (endothermic) and comparing the result with well established data from the literature for the corresponding reaction.

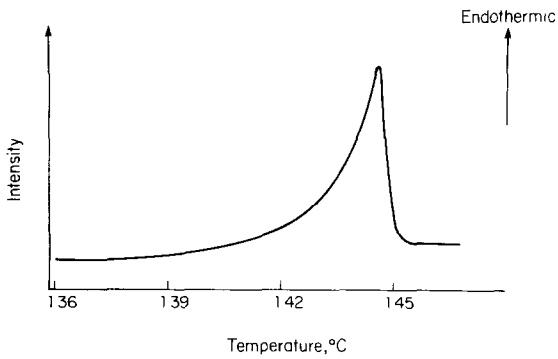


Fig. 1. Differential scanning calorimetry of enalapril maleate.

Results and Discussion

X-ray diffraction studies gave the first indication of the possibility of polymorphic forms of enalapril maleate. Subsequent studies were carried out to confirm or deny this possibility. The data suggested that two polymorphic forms existed. The form first prepared (ethyl acetate/methanol crystallized) was designated as Form I and the form subsequently prepared (ethyl acetate/methanol crystallized and water slurried) was designated as Form II. Presented below are the data accumulated by each of the experimental techniques employed to support or deny the existence of two distinct polymorphic forms.

DSC

DSC runs were obtained using 4–6 mg samples. A typical DSC is shown in Fig. 1. DSC data are listed in Table 1. The mean $\pm$S.D. values for T_0 (uncorr.) and heats of fusion (ΔH_f) for the Form I samples are 144.0 ± 0.2°C and 14.0 ± 0.5 kcal/mol. The corresponding mean $\pm$S.D. values for the Form II samples are 142.3 ± 0.3°C and 13.6 ± 0.4 kcal/mol. The differences in the T_0 (uncorr.) and ΔH_f for the two forms are considered to be small and within experimental error.

Phase solubility analysis

Extrapolated solubilities of approximately 2.6 and 2.7 mg/ml in acetonitrile/ isopropyl acetate (1/1, by volume) were obtained for Form I and Form II, respectively, (Table 1) and were not significantly different among these two forms.

X-Ray powder diffraction (XRPD)

XRPD patterns corresponding to Form I and Form II are shown in Figs. 2 and 3, respectively.

TABLE 1

DSC AND PHASE SOLUBILITY ANALYSES [a]

Sample	Form	DSC			Phase solubility	
		T° (uncorr.) (°C)	ΔH_f (kcal/mol)	Mol. % impurity	Extrapolated (mg/ml)	% Slope (%)
1	I	143.4 ± 0.1	14.5 ± 0.2	0.9 ± 0.2	2.6 ± 0.1	0.9 ± 0.2
2	I	146.2 ± 0.2	14.6 ± 0.3	0.6 ± 0.2	2.6 ± 0.1	0.3 ± 0.5
3	I	143.3 ± 0.4	13.5 ± 1.5	1.9 ± 0.6	2.6 ± 0.1	1.7 ± 0.4
4	I	142.9 ± 0.1	13.2 ± 0.1	0.8 ± 0.2	2.4 ± 0.1	1.4 ± 0.3
5	II	144.7 ± 0.1	14.1 ± 0.4	1.4 ± 0.2	2.7 ± 0.1	0.4 ± 0.3
6	II	142.7 ± 0.1	13.2 ± 0.0	1.1 ± 0.7	–	–
7	II	141.3 ± 0.3	13.3 ± 0.3	1.3 ± 0.0	–	–
8	II	140.4 ± 0.5	13.7 ± 0.7	2.4 ± 0.2	–	–

[a] Data are expressed as average of duplicate measurements $\pm$ S.D.

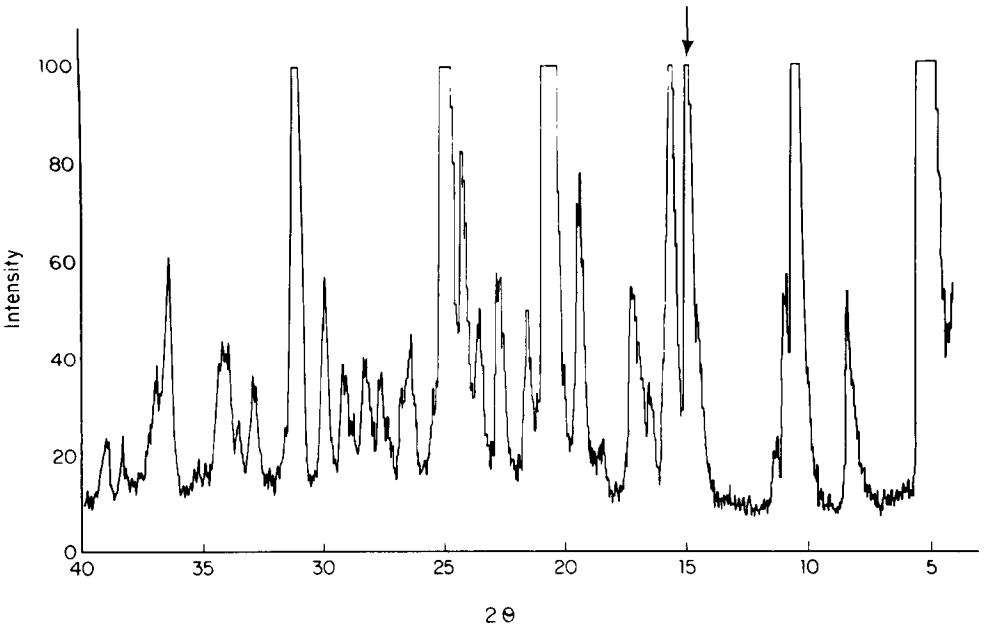


Fig. 2. Powder X-ray diffraction pattern of Form I.

There are minor shifts centered at around 19.0 and 22.4° 2θ. However, an extra peak is present at 14.7° 2θ for Form I and at 13.0° 2θ for Form II. By employing sample spinning techniques, these extra peaks were demonstrated not to be an artifact caused by preferred orientation.

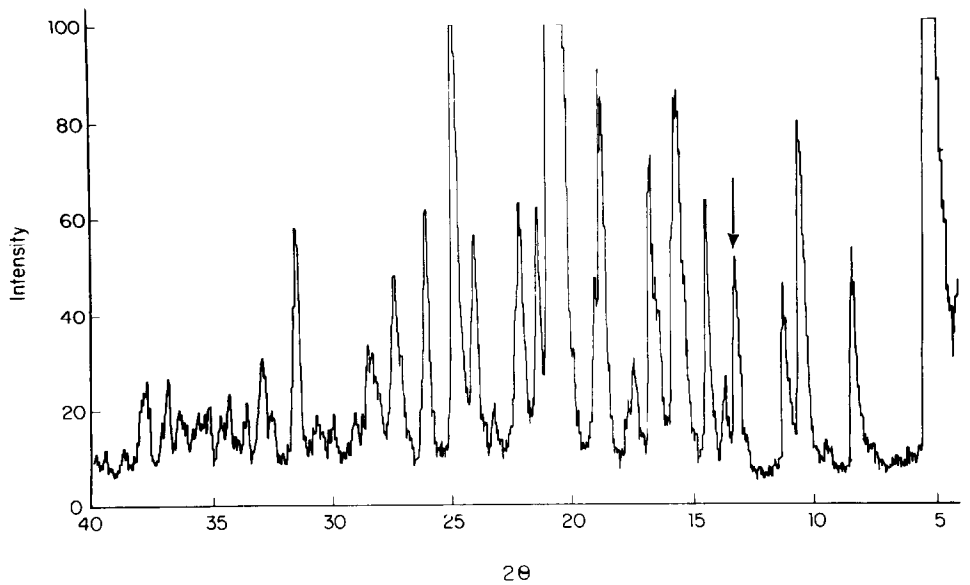


Fig. 3. Powder X-ray diffraction pattern of Form II.

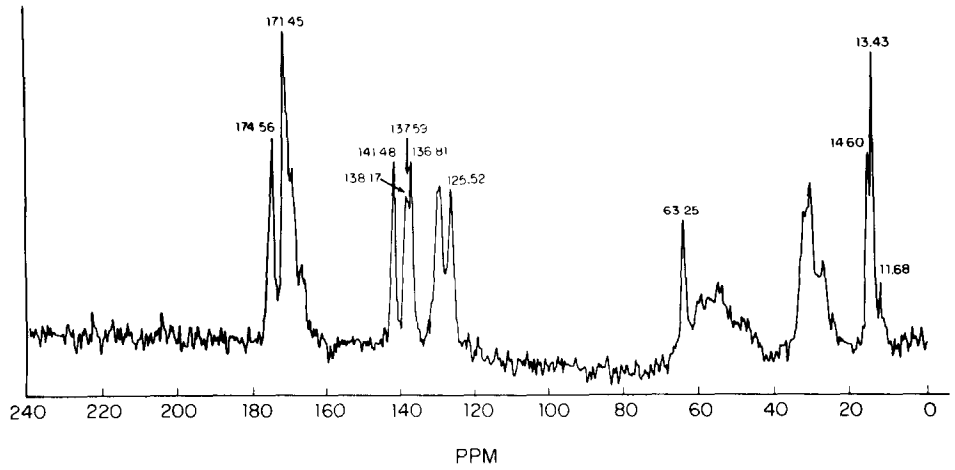


Fig. 4. Solid-state carbon-13 NMR of Form I.

NMR

Application of solid state carbon-13 NMR to pharmaceutical studies includes determination of polymorphs (Ripmeester, 1980; Atalla et al., 1980; Byrn et al., 1985).

The results of solid state carbon-13 NMR in the present studies clearly distinguish the two forms of enalapril maleate. Figs. 4 and 5 represent the spectra of Form I and Form II, respectively. Major differences correspond to the ethyl ester methyl and maleate carbon signals (in the 11–13 ppm and 137–138 ppm, respectively). Other differences occur with respect to intensity and/or line-broadening involving aromatic carbons at ~ 129 ppm and the proline C_5. The latter does not manifest itself clearly in the Form I sample, although a fairly sharp peak at ~ 47 ppm is thought to be due to this carbon site for Form II.

Infrared and Raman

Quantitative (Ebert et al., 1952; Kendall, 1953; Smakula et al., 1957) as well as qualitative (Ebert et al., 1952; Kendall, 1953; Cleverley et al., 1959; Mesley et al., 1968; Chapman et al., 1968) identification of polymorphism by IR spectroscopy has been reported.

The infrared spectra of the various samples of enalapril maleate are all *nearly* identical, showing bands of similar intensity at similar positions throughout the mid-IR (400–4000 cm^{-1}). How-

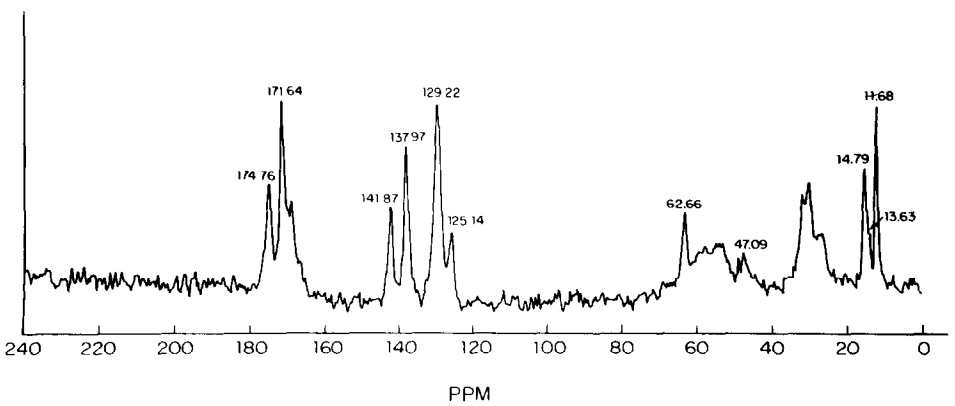


Fig. 5. Solid-state carbon-13 NMR of Form II.

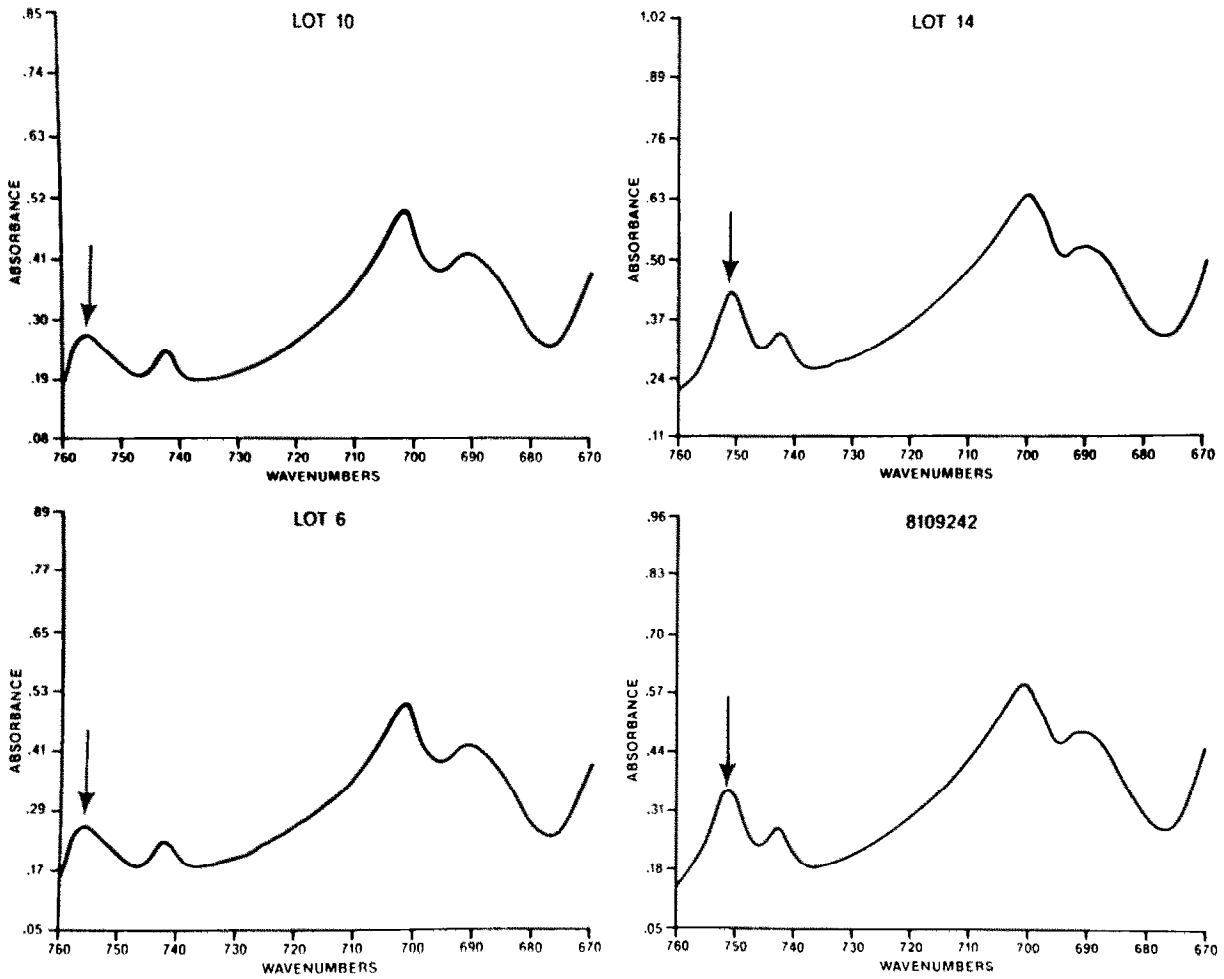


Fig. 6. Comparison of the infrared absorption spectra of various lots of enalapril maleate in the region where the greatest mid-IR spectral differences occur. The largest difference (5 cm^{-1}) occurs at 750–760 cm^{-1}.

ever, close examination of the spectra reveals that they can be separated into two groups. The largest spectral difference between the two groups is a 5 cm^{-1} difference in one band, which is likely due to an out-of-plane hydrogen bond of the phenyl ring (751 vs 756 cm^{-1}). Spectra of the two groups in this region are shown in Fig. 6. There are other small (1–2 cm^{-1}) frequency, intensity and band shape difference throughout the mid-IR spectrum.

Small shifts observed in the IR bands are indicative of the weak forces involved. This therefore suggests low frequency vibration (usually less than 200 cm^{-1}). Laser Raman spectroscopy was, therefore, appropriate to obtain information regarding low frequency vibrations. The low frequency Raman spectroscopic technique has been applied recently to study the pressure-induced phase transition in 4,4′-dichlorobenzophenone (Kirin et al., 1982).

The Raman spectra of the two groups are also nearly identical except in the low frequency region of the spectrum. The low frequency Raman spectra of lots 6 and 10 (Form I) are dominated by an intense, sharp band at about 24 cm^{-1}. This band is extremely weak or absent in lot 14 and sample 8109242 (Form II). The difference between the

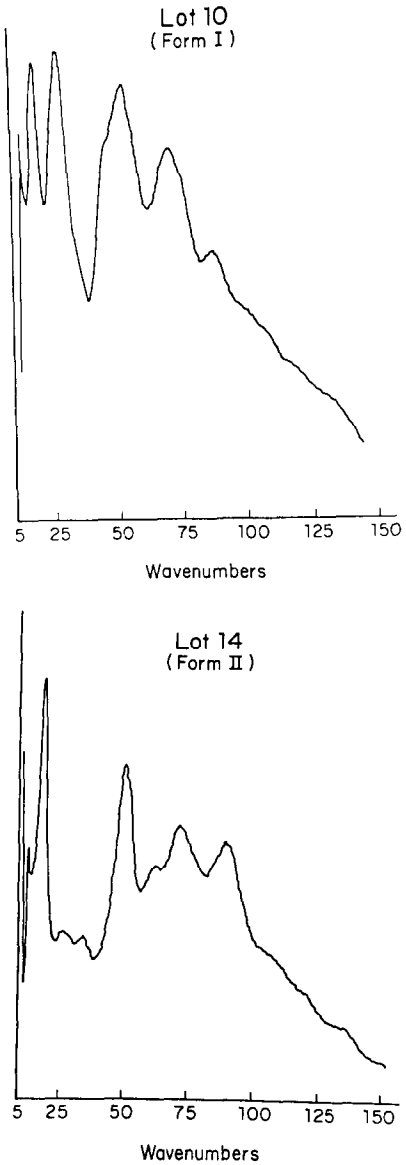


Fig. 7. Anti-Stokes Raman spectra of Forms I and II of enalapril maleate. The lowest frequency feature at 14 cm^{-1} is a laser plasma line.

two groups is illustrated by the anti-Stokes spectra shown in Fig. 7. The Stokes spectra show similar phenomena in the low frequency region (except, of course, for the plasma line at 14 cm^{-1} in the anti-Stokes spectrum).

The two groupings found by Raman spectroscopy are the same as that found by infrared. That is, samples with the strong Raman band at 24 cm^{-1} have an IR band at 756 cm^{-1}, while those without an intense band at 24 cm^{-1} have an IR band at 751 cm^{-1}. The 24 cm^{-1} band is most likely due to a lattice vibration.

The minor differences seen between the infrared and Raman spectra of the two groups of samples except at low frequencies are consistent with the existence of two polymorphs having very similar molecular conformations but different crystal packing. These kinds of subtle spectral differences are common for polymorphs of simple aromatic molecules where only weak electrostatic and van der Waals intermolecular forces are present. However, it is somewhat surprising that a molecule with several charged sites and sites capable of hydrogen bonding would show similar behavior. Other evidence that the crystal packing of the two polymorphs is dominated by weak nonbonding interaction include the high carboxylic acid carbonyl stretch at ~ 1760 cm^{-1} characteristic of a free, not a hydrogen-bonded acid.

Heats of solution

The results obtained for the heats of solution of the four samples (two for each form of enalapril maleate) in acetone and methanol are given in Table 2. All heats were endothermic.

As polymorphs are solid compounds that are identical chemically and differ only in crystal

TABLE 2

HEATS OF SOLUTION OF ENALAPRIL MALEATE

Solvent	ΔH_S(kcal/mol)	
	Form I	Form II
Methanol	8.720	9.189
	8.728	9.128
	8.587	9.208
	8.647	9.227
	8.710	–
Mean ± S.D.	8.678 ± 0.060	9.188 ± 0.026
Acetone	14.20	14.98
	14.27	14.81
	14.14	14.97
	14.27	14.94
	–	14.84
Mean ± S.D.	14.22 ± 0.06	14.91 ± 0.07

structure, the individual heats of solution ΔH_S^I and ΔH_S^{II} will depend on the solvent used. The difference in heats of solution, however, is independent of the solvent and will be equal to the difference in lattice energy of the solids. The heats of transition (ΔH_T) of Form II to Form I may be calculated as follows:

$$\Delta H_T = \Delta H_S^{II} - \Delta H_S^I \quad (1)$$

The values obtained for ΔH_T are 0.69 ± 0.13 kcal/mol in acetone and 0.51 ± 0.09 kcal/mol in methanol. The good agreement as to the difference between them, ΔH_T (independent of solvent used), provides firm evidence that they are identical chemically but differ in crystal structure.

Since Form II is more endothermic than Form I upon dissolving, it is clearly the more thermodynamically stable form. The difference in heat of solution for a given form in two different solvents is expected to be the same for both forms. In the case of enalapril maleate this was shown to be the case with values of 5.54 ± 0.12 kcal/mol for Form I, and 5.72 ± 0.11 kcal/mol for Form II with an average of 5.63 ± 0.11 kcal/mol. This is a measure of the difference in the heat of solvation of enalapril maleate in acetone and methanol; i.e. the heat of solvation in methanol is 5.63 kcal/mol more exothermic than in acetone. This value is in the expected range for the formation of a single hydrogen bond.

TABLE 3

DISSOLUTION DATA

Enalapril maleate formulation	Crystal form	Potency (mg)	Percent dissolved (Av.) at 30 min
Capsules	II	2.5	89
	I	2.5	100
	I and II	2.5	101
	I	2.5	96
	I and II	20	82
	I	20	99
	II	20	95
	I	20	92
Tablets	I	10	100
	II	10	99
	I	10	99
	I and II	10	98
	I	40	103
	I and II	40	102
	II	40	96

Dissolution

Dissolution data from enalapril maleate formulations of capsules as well as tablets using crystalline Form I or Form II or combinations of both were obtained and are shown in Table 3. No significant differences in dissolution data were seen for these formulations. This supports the conclusion that for these forms, control of the crystal form is not essential to guarantee the dissolution performance of the dosage form.

Conclusion

A number of high resolution spectroscopic methods have provided a powerful means to identify polymorphic forms of enalapril maleate. Heats of solution determinations have also distinguished the two crystalline forms. The calorimetric data for solutions have also shown Forms I and II to be very similar in energy, with Form II being slightly more stable than Form I by 0.6 kcal/mol. In vitro dissolution experiments demonstrate similar dissolution data using capsule and tablet formulations containing either one or both polymorphic forms.

Acknowledgements

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References

Atalla, R.H., Gast, J.C., Sindorf, D.W. Bartuska, V.J. and Maciel, G.E., ^{13}C NMR spectra of Cellulose polymorphs, J. Am. Chem. Soc., 102 (1980) 3249–3251.

Biles, J.A., Some crystalline modifications of the *tert*-butylacetates of prednisolone and hydrocortisone, J. Pharm. Sci., 52 (1963) 1066–1070.

Byrn, S.R., Solid-State Chemistry of Drugs, Academic Press, New York, 1982, p. 79.

Byrn, S.R., Gray, G., Pfeiffer, R.R. And Frye, J., Analysis of solid-state carbon-13 NMR spectra of polymorphs (Benoxaprofen and Nabilone) and pseudopolymorphs (Cefazolin). J. Pharm. Sci., 74 (1985) 565–568.

Chapman, J.H., Page, J.E., Parker, A.C., Rogers, D., Sharp, C.J. and Staniforth, S.E., Polymorphism of cephaloridine. J. Pharm. Pharmacol., 20 (1968) 418–429.

Cleverley, B. and Williams, P.P., Polymorphism in substituted barbituric acids. Tetrahedron, 7 (1959) 277–288.

Ebert, A.A. and Gottlieb, H.B., Infrared spectra of organic compounds exhibiting polymorphism. J. Am. Chem. Soc., 74 (1952) 2806–2810.

Haleblian, J. and McCrone, W., Pharmaceutical applications of polymorphism. J. Pharm. Sci., 58 (1969) 911–929.

Haleblian, J., Characterization of habits and crystalline modification of solids and their pharmaceutical applications. J. Pharm. Sci., 64 (1975) 1269–1288.

Kendall, D.N., Identification of polymorphic forms of crystals by infrared spectroscopy. Anal. Chem., 25 (1953) 382–389.

Kirin, D. and Pawley, G.S., Low-frequency Raman spectrum and pressure-induced phase transition of crystalline 4,4′-dichlorobenzophenone. Chem. Phys. Lett., 85 (1982) 298–301.

Mesley, R.J., The polymorphism of sulphathiazole. J. Pharm. Pharmacol., 23 (1971) 687–694.

Mesley, R.J., Clements, R.L., Flaherty, B. and Goodhead, K., The polymorphism of phenobarbitone. J. Pharm. Pharmacol., 20 (1968) 329–340.

Ripmeester, J.A., Application of solid state ^{13}C NMR to the study of polymorphs, clathrates and complexes. Chem. Phys. Lett. 74 (1980) 536–538.

Smakula, E., Gori, A. and Wotiz, H., Effects of polymorphism and influence of sample preparation on the infra-red spectrum of estradiol-17β. Spectrochim. Acta, 9 (1957) 346–356.

Trivedi, J., Shell, J.W. and Biles, J.A., Some physical and crystallographic properties of the ouabain hydrates. J. Am. Pharm. Ass., Sci. Edn, 48 (1959) 583–587.