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Variable-temperature X-ray powder diffraction analysis of the crystal transformation of the pharmaceutically preferred polymorph C of mebendazole

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Abstract

Mebendazole is a common benzimidazole anthelmintic that is water insoluble. It is reported to exist in three different polymorphic forms in the solid state, i.e. polymorph A, B and C. Form C is the pharmaceutically preferred form because of its increased aqueous solubility. This paper deals with the use of variable-temperature X-ray powder analysis (VTXRPD) to study the transformation of Form C. Results showed that Form C was stable and transformed to the more stable polymorph A at high temperature (>180 °C). This transformation is a first-order process with activation energy of 238 ± 16 kJ/mole. Further studies showed that compression did not cause any significant changes in the crystal structure of polymorph C.

Keywords: Mebendazole; Polymorph transformation; Variable-temperature X-ray powder diffraction

1. Introduction

Mebendazole (Fig. 1) is a common benzimidazole anthelmintic used in the treatment of ascariasis, uncinariasis, oxyuriasis, trichuriasis and more than one worm infection at a time. It inhibits glucose uptake in the parasite, resulting in immobilization and death. It is a white to slightly yellow powder; insoluble in water, acid solutions, alcohol, ether and chloroform; freely soluble in formic acid [1–3]. Its chemical designation is 5-benzoyl-2-benzimidazolecarbamic acid methyl ester. This anthelmintic drug can exist as three distinct crystal forms in the solid state, i.e. polymorphs A, B and C [1–4]. The solubility of these forms in physiological media is Form B > Form C > Form A [5]. Based on these differences Form C is pharmaceutically preferred since its solubility is sufficient enough to ensure optimal bioavailability without the possible toxicity of the more soluble Form B [5,6]. Ren et al. [7] found that Form A had no anthelmintic activity when alone or when present above 30% in polymorphic mixtures.

The method of choice to differentiate between the polymorphic forms is IR spectroscopy [2,5,8,9]. However, with this technique it is difficult to quantify polymorphic mixtures [10]. X-ray powder diffraction (XRPD) analysis based on diffraction peak angles and corresponding intensities is often preferred to quantify polymorphic forms [11,12]. Himmelreich et al. [1] and Ma and Hua [2] reported the X-ray diffractograms and thermal properties of mebendazole crystal forms. Thermal stability analysis reported in these studies also showed that Form B and Form C are transformed to Form A upon heating at temperatures above 210 and 170 °C, respectively [1].

Abbreviations: XRPD, X-ray powder diffraction; VTXRPD, variable-temperature X-ray powder diffraction; DSC, differential scanning calorimetry; IR, infrared spectroscopy; DRIFTS, diffuse reflectance infrared spectroscopy; HPLC, high-performance liquid chromatography

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In this present study we looked at the effect of temperature on the transformation of Form C by combining thermal analysis with XRPD analysis. Variable-temperature X-ray powder diffraction (VTXRPD) analysis has been used to characterize complex pharmaceutical solid-state reactions including crystal transformations [12,13]. This technique is a powerful tool that is used to probe such reactions since it permits simultaneous quantification of multiple solid phases [14,15]. XRPD was also used to determine the effect of heat generated during compression on the transformation of Form C. The results of this study are important since currently there are no official methods to characterize the crystal forms of mebendazole.

2. Experimental

2.1. Materials and preparation of polymorphs

Mebendazole Fig. 1 was obtained from Spectrum Quality Products Inc. (Batch number: OH0506, Gardena, CA, USA). All solvents used were of analytical grade and were obtained from Spectrum Chemical Corp. (Gardena, CA, USA). The mebendazole polymorphs were prepared by recrystallization [1,2]. Form A was recrystallized from glacial acetic acid, Form B from chloroform and Form C from methanol. The purity of the powders was between 99% and 101% as determined using the methods described in the USP including IR spectroscopy and HPLC analysis [16]. Mean assay for Form A = 99.7 ± 1.2%; Form B = 99.1 ± 0.9%; Form C = 98.6 ± 1.4% (n = 6).

2.2. Characterization of polymorphs

The polymorphs were characterized by X-ray powder diffractometry (XRPD), diffuse reflectance infrared spectroscopy (DRIFTS), and differential scanning calorimetry (DSC). DSC traces were recorded with a DSC 2920 modulated DSC (TA Instruments, New Castle, DE, USA). Indium (melting point 156.6 °C) and tin (melting point 231.9 °C) were used to calibrate the instruments. A mass, not exceeding 3.0 mg, was measured into aluminium pans with or without a small pinhole in the lid. DSC curves were obtained under a nitrogen purge of 20 mL/min at a heating rate of 10 K/min. Heating rates of 5–20 °C were used to examine changes in melting points and dehydration peaks. Melting temperatures were determined as extrapolated onset temperatures, defined as the point of transition, being the point of intersection between the base line and the DSC endothermal melting effect, which gives the most reproducible value, experimentally independent of the operator.

Ambient XRPD determinations were measured using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromatic; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025°, step time 1.0 s). Approximately 300 mg samples were weighed into aluminium sample holders, taking care not to introduce a preferential orientation of crystals.

DRIFTS spectra were recorded on a Nexus^{TM} 470 spectrophotometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000–400 cm^{-1} with the Avatar Dif-

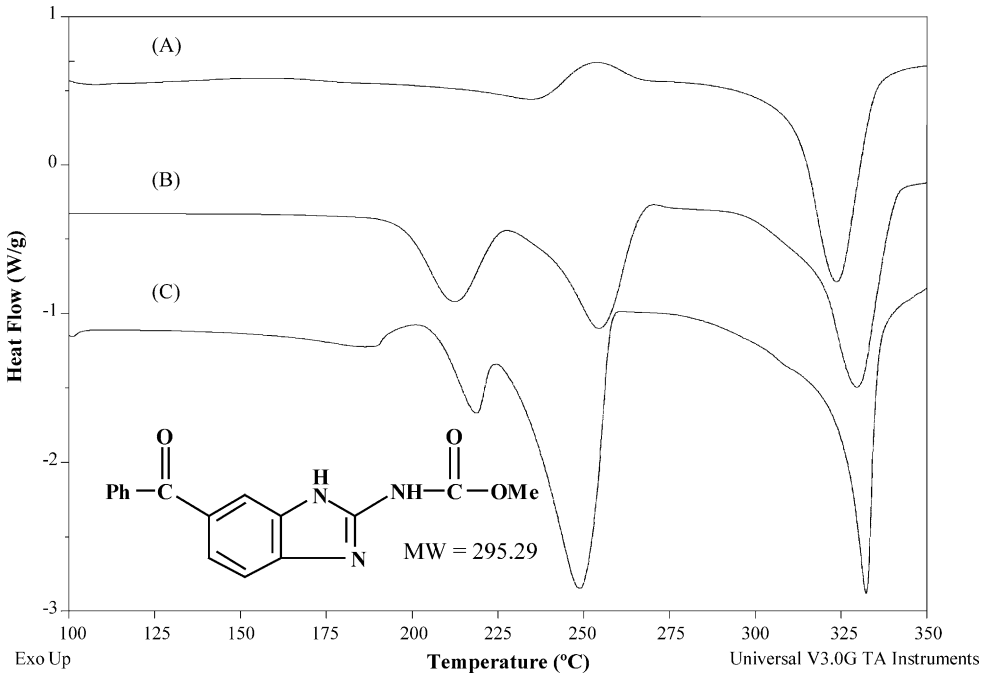

Fig. 1. DSC thermograms of the three mebendazole polymorphs.

fuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling. DSC thermograms were recorded with a modulated DSC 2920 (TA Instruments Inc., New Castle, DE, USA). Samples weighing approximately 3–5 mg were heated in closed aluminium crimp cells at a rate of 10 °C/min under nitrogen gas flow of 20 mL/min.

2.3. Variable-temperature X-ray powder analysis

Variable-temperature X-ray powder diffraction patterns were recorded with an Anton Paar TTK 450 low-temperature camera (Anton Paar, Austria) attached to the Bruker D8 Advance diffractometer (Bruker, Germany). A heating rate of 10 °C/min was used during all of these determinations. The isothermal measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025°, step time 1.0 s). Approximately 150 mg samples were weighed into the sample holder, taking care not to introduce a preferential orientation of crystals.

2.4. Kinetics of polymorph transformation

The method used in this study to determine the solid-state kinetics of crystal transformations involved the isothermal measurement of changes in the intensities of a characteristic diffraction peak of polymorph C (4.9°2θ) and polymorph A (7.7°2θ) at 175, 185 and 190 °C as a function of time [11]. The rate of decrease in intensity of the characteristic peak of a certain phase as a function of time is an indication of the kinetics of the disappearance and transformation of this phase. Likewise, the concurrent increase of characteristic peak intensity of a second phase as a function of time quantified the kinetics of the appearance of the new crystalline phase [11].

Table 1
Main characteristic IR peaks at corresponding wavenumbers (cm^{-1}) of mebendazole polymorphs A, B and C [1,5] and the crystal form obtained by heating Form C at 210 °C

Wavenumbers (cm^{-1})			
Form A	Form B	Form C	Heated Form C
3370	3340	3410	3369
1730	1700	1720	1731
1640	1650	1645	1639
640	–	660	642

2.5. Effect of compression

To study further the effect of powder processing procedures associated with the possible increase in temperature on the crystal transformation of Form C, the transformation during compression was also monitored. Changes were characterized by XRPD and DSC data obtained before and after compression. Tablets composed of 350–450 mg of drug were compressed at 306 MPa for 15 s with an automated hydraulic press (Carver Inc., Wabash, Indiana, USA) using 1.36 cm stainless steel dies.

3. Results and discussion

It is customary to use the IR absorption spectra of the various polymorphic forms of mebendazole for identification because there are characteristic differences in the shape and intensities of some of the major absorption bands [1,5,8,9]. In particular the carbonyl stretching frequency (1700–1730 cm^{-1}) and the –NH stretching frequency (3340–3410 cm^{-1}) are different in each form and are used to identify each polymorph [2,4]. The characteristic peaks at corresponding wavenumbers (cm^{-1}) of mebendazole polymorphs A, B and C prepared in this study and that of the crystal form obtained by heating polymorph C are shown in Table 1. IR data in combination with XRPD (Table 2) and DSC (Fig. 1) results clearly identified the crystal forms and showed that the form obtained by heating polymorph C was indeed polymorph A. However, the IR spectrum of

Table 2
Main XRPD peak angles d (Å) and intensity ratios (I/I_0) of mebendazole Form A, B and C, and the crystal form produced by heating Form C at 210 °C

Main peaks	Form A		Form B		Form C		Heated Form C	
	d (Å)	I/I_0 (%)	d (Å)	I/I_0 (%)	d (Å)	I/I_0 (%)	d (Å)	I/I_0 (%)
1	11.52	100	4.65	100	4.48	100	11.52	100
2	6.13	25	9.34	85	3.34	73	6.13	17
3	5.13	70	3.64	68	17.91	72	5.12	69
4	4.87	19	4.13	61	3.60	56	4.87	15
5	4.49	13	14.62	60	5.45	51	4.48	10
6	4.35	11	3.54	51	3.09	36	4.33	9
7	3.84	38	7.09	49	4.59	32	3.83	22
8	3.78	47	4.22	47	4.89	30	3.78	34
9	3.61	34	3.94	46	4.16	28	3.61	23
10	3.53	23	3.09	43	7.19	28	3.53	17

a mixture of mebendazole polymorph A and polymorph C exhibited characteristic absorption bands at wavenumbers of 3368, 1725, 1643 and 644 cm^{-1}, characteristic of all three forms. Care should therefore be taken when using IR spectral data alone to identify mebendazole polymorphic forms.

3.1. Effect of increased temperature on mebendazole polymorph C

The DSC thermograms of the polymorph C exhibited three thermal events: a small endothermic/exothermic event (195 °C) followed by small endotherm at 225 °C and two sharply defined endotherms (Fig. 1). The first is a sharply defined endotherm at 253 °C, which is followed by a second and final melting endotherm at 330 °C. The DSC thermogram of Form B is characterized by three broad endotherms at 220, 263 and 330 °C, respectively. In the thermogram of Form A there is only an endothermic/exothermic transition at 250–255 °C and the final melting endotherm at 330 °C.

3.2. Variable-temperature X-ray powder diffractometry

Variable-temperature XRPD analysis clearly showed that mebendazole polymorph C was stable between room temperature and ±179 °C (Fig. 2). No crystal transformation seems to occur between these temperatures. Further increases in temperature lead to the transformation of polymorph C to a different crystal form altogether (Fig. 2). This transformation of polymorph C to a different crystal form was complete at ±225 °C (Fig. 2). The small endothermic/exothermic event

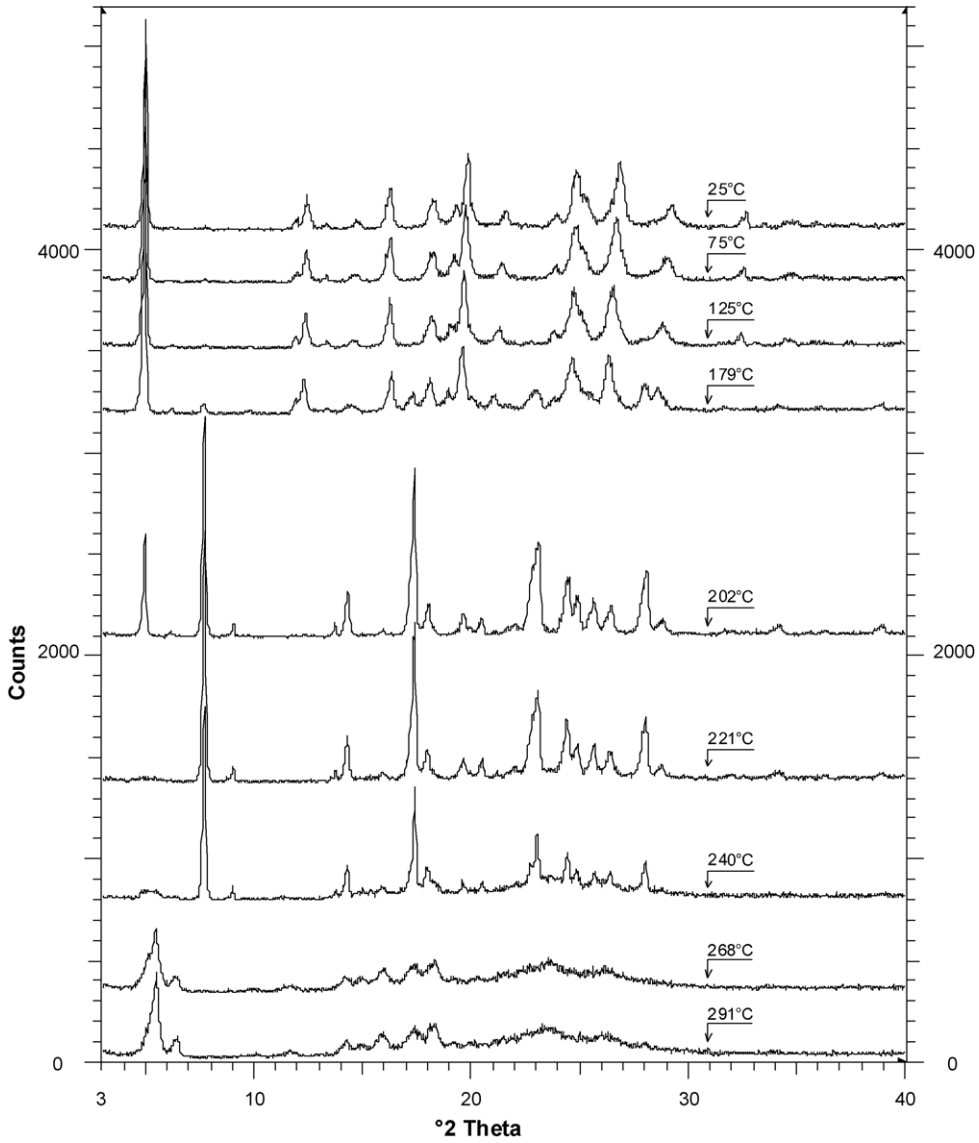

Fig. 2. VTXRPD patterns of mebendazole Form C characterizing specific crystal changes.

at ±195 °C in the DSC thermogram (Fig. 1) likely indicated the start of these changes in the crystal structure of polymorph C. The fact that this change was small likely indicates that this process was an internal rearrangement of the crystal structure.

The crystal structure (XRPD pattern) of this crystal form obtained by heating polymorph C to >200 °C (Fig. 2) was comparable to the crystal structure (XRPD pattern) of mebendazole polymorph A [1,5]. The XRPD results, as shown in Table 2, indicated that polymorph C transformed to polymorph A upon heating between 200 and 225 °C. These XRPD results were confirmed by DSC analysis. The transformation of mebendazole polymorph C to polymorph A (at ±200–225 °C) probably occurs via the formation of a mixture of polymorphs A and C (Fig. 2). This was suggested by the XRPD pattern (202 °C), which might represent a mixture of these two polymorphic forms. Fig. 2 furthermore showed that the melting and degradation process of mebendazole polymorph A started at temperature above 240 °C. This degradation process was clearly illustrated by changes in the crystal structure observed at these temperatures and by the formation of an amorphous-like crystal form at higher temperatures (Fig. 2).

A mixture of mebendazole polymorph A and polymorph C was also identified during comparative raw material analysis (Fig. 3, 25 °C) [4]. The XRPD pattern of this mixture exhibited diffraction peaks that were a combination of the patterns of polymorph A (Fig. 2, 221 °C) and polymorph C (Fig. 2, 25 °C). This mixture of polymorphs A and C also exhibited DSC endotherms at 263 and 330 °C, consistent with that of a mixture of polymorphs A and C. Variable-temperature XRPD patterns obtained for this mixture of polymorphs A and C indicated that this mixture was stable from room temperature to approximately 195 °C (Fig. 3). A further increase in temperature leads to transformation of the remaining amount of polymorph C to the more stable polymorph A (Fig. 2).

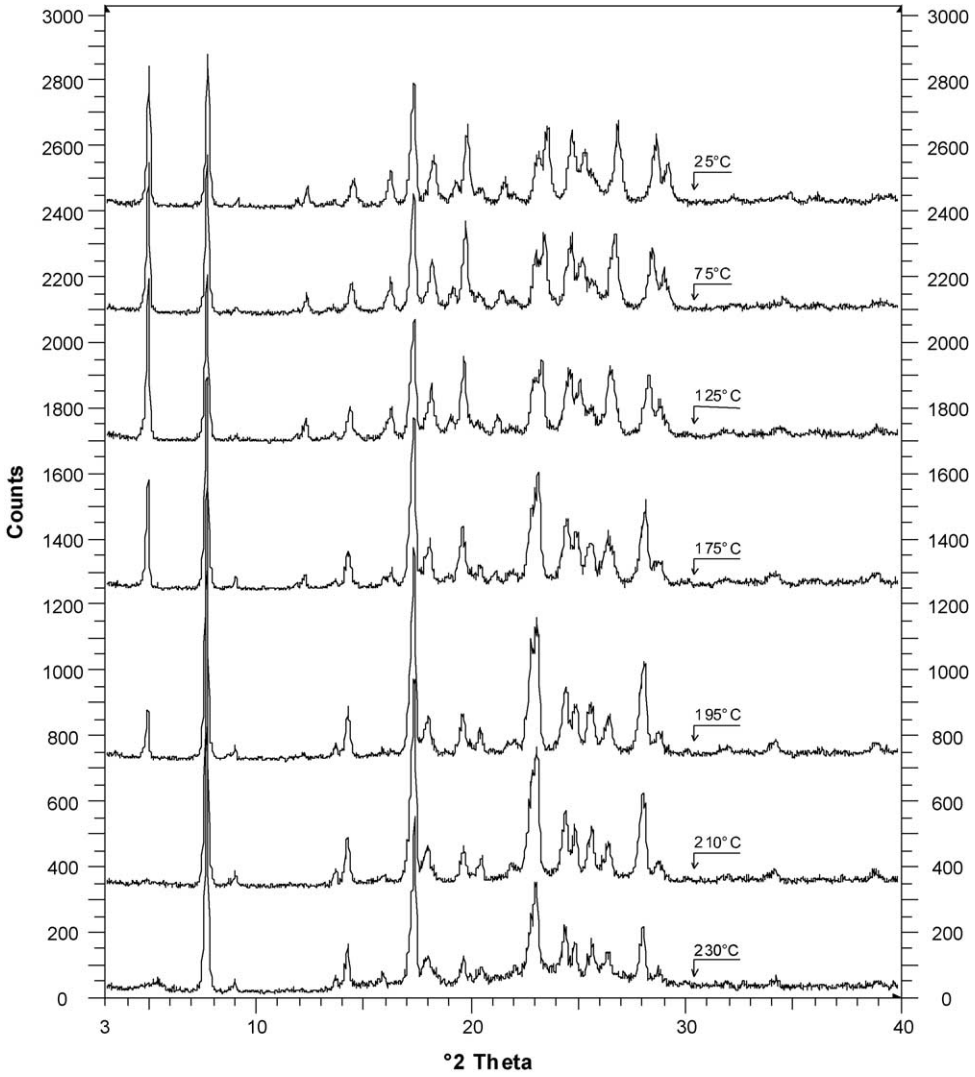

Fig. 3. VTXRPD patterns of a mixture of mebendazole Form A and Form C.

3.3. Kinetics of the transformation of Form C to A at increased temperature

To determine the kinetics of the transformation of mebendazole polymorph C to polymorph A at increased temperature, the disappearance of a characteristic diffraction peak of polymorph C (4.9°2θ) and the appearance of a characteristic diffraction peak of polymorph A (7.7°2θ) was followed as a function of time at 175, 185 and 190 °C. Scans were obtained over an angular range of 4–10°2θ and the theoretically maximum peak intensity values $(Ii_1)_0$ determined at 25 °C for polymorph C and at 215 °C for polymorph A. These are the temperatures at which pure polymorph C and A are theoretically present.

The theoretical basis of quantification of crystalline phases in a mixture is that in a two-component mixture the intensity of line I of component 1 (Ii_1) is related to its mass fraction x_1. According to Eq. (1) [11]:

$$\frac{Ii_1}{(Ii_1)_0} = \frac{x_1\mu_1^*}{x_1(\mu_1^* - \mu_2^*) + \mu_2^*} \quad (1)$$

where $(Ii_1)_0$ is the intensity of line I in a sample consisting only of component 1, and μ_1^* and μ_2^* are the mass attenuation coefficients of component 1 and 2, respectively. The mass attenuation coefficient is defined as the linear attenuation coefficient divided by the density of the substance (cm^2/g). For a given incident X-ray energy, the mass attenuation coefficient is independent of the physical and chemical state of the absorber. Thus, the mass attenuation coefficient of a compound or mixture of elements can be calculated by taking the weighted sum of the mass attenuation coefficients for each element, where weight is assigned according to the fraction of the element contributing to the compound. In the case of mebendazole polymorph C and polymorph A $\mu_1^* \cong \mu_2^*$ and Eq. (1) approximated to Eq. (2) [11]:

$$\frac{Ii_1}{(Ii_1)_0} = x_1 \quad (2)$$

Graphs of mass fractions of the different polymorphic phases (polymorphs C and A) as a function of time were plotted for each temperature (for example, at 175 °C, Fig. 4). Results showed that in the temperature range (175–190 °C) the disappearance of polymorph C as a function of time was a first-order process. This was evident from the straight lines and linearity factors of $R^2 \geq 0.900$ obtained from natural logarithm graphs of peak intensity as a function of time (Fig. 5). The reaction rate constants at each temperature could be calculated from these graphs (Fig. 5). The Arrhenius plot (Fig. 6) of the rate constants at each isothermal temperature produced a straight line with a linearity factor of $R^2 = 0.994$ and the activation energy (E_a) for the transformation of polymorph C to A was calculated to be 238 ± 16 kJ/mole from the slope of the Arrhenius plot.

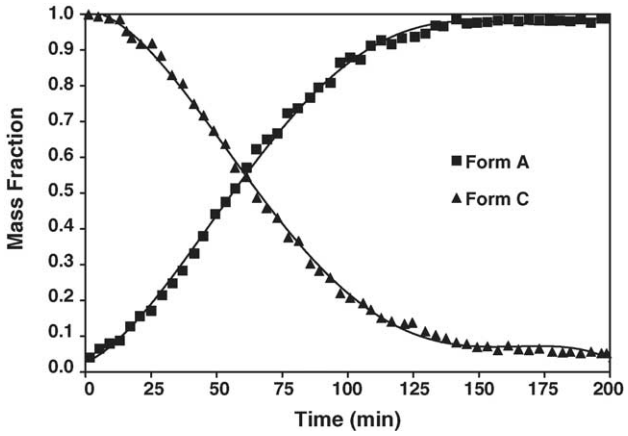

Fig. 4. Mass fraction of mebendazole Form C and Form A as a function of time following isothermal XRPD analysis at 175 °C.

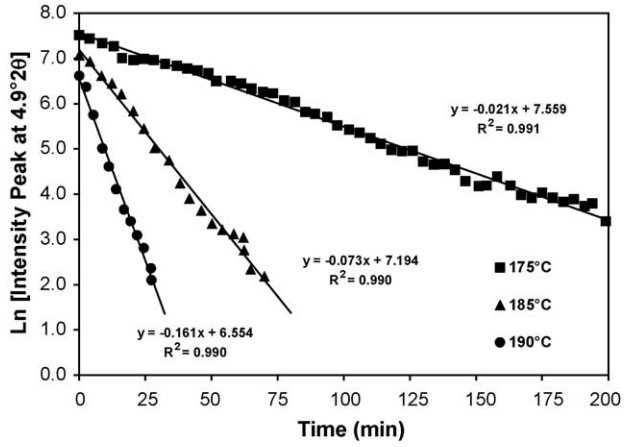

Fig. 5. Natural logarithm of the peak intensity of mebendazole Form C as a function of time at 175, 185 and 190 °C.

3.4. Effect of compression

Many pharmaceutical processing procedures involve an increase in temperature. One such process is the compression

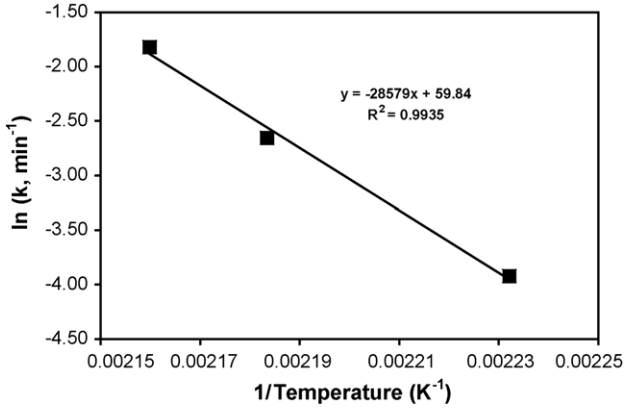

Fig. 6. Arrhenius plot of the transformation of mebendazole polymorph C to polymorph A at high temperatures (175–190 °C).

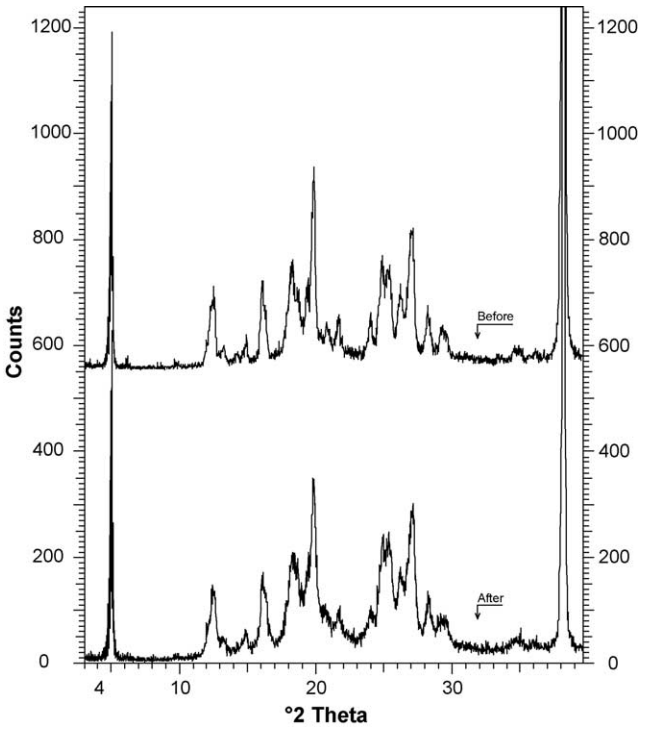

Fig. 7. XRPD patterns of mebendazole Form C before and after compression.

of powders into tablets. To see if compression increased the transformation of Form C to A, XRPD patterns were taken of samples of Form C were taken before and after compression. Fig. 7 shows that compression did not lead to any significant changes in the crystal structure of Form C, since the XRPD patterns before and after compression were comparable. Compression did cause a slight decrease in XRPD peak intensity at corresponding 20 values of 4.9° for polymorph C and 7.7° for polymorph A. This could be due to sample differences, compressed versus powdered. DSC thermograms obtained before and after compression confirmed that Form C stayed intact during compression.

4. Conclusion

Variable-temperature X-ray powder diffractometry showed that the pharmaceutically preferred polymorph C of mebendazole was stable between room temperature and approximately 179 °C. A further increase in temperature (to between 205 and 220 °C) leads to the transformation of polymorph C to the more stable polymorph A. Isothermal studies at high temperature characterized this transformation process as a first-order process with an activation energy (E_a) for the transformation of polymorph C to A equal to 238 ± 16 kJ/mole. This means that mebendazole form C is very stable under ambient conditions. In addition, compression also did not cause significant changes in the crystal structure of polymorph C.

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