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Thermal methods for evaluating polymorphic transitions in nifedipine

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Abstract

The thermal behaviour of nifedipine was studied with the view to understand the various phase transitions between its polymorphs. The focus was on polymorph identification, accompanying morphological changes during crystallization and the nature of the phase transformations. These features were compared to the complexity of the crystallization mechanisms, studied by dynamic differential scanning calorimetry (DSC) heating techniques. DSC, thermogravimetry (TG) established the temperature limits for preparation of amorphous nifedipine from the melt. DSC studies identified that metastable form B, melting point ∼163 °C, was enantiotropically related to a third modification, form C, which existed at lower temperatures. Form C converted endothermically to form B at ∼56 °C on heating and was shown by hot stage microscopy (HSM) to be accompanied by morphological changes. Modulated temperature differential scanning calorimetry (MTDSC) showed discontinuities in the reversing heat flow signal during crystallization of amorphous nifedipine (from ∼92 °C) to form B, which suggested that a number of polymorphs may nucleate from the melt prior to form B formation. Identification of the number of nifedipine polymorphs included the use of combined DSC–powder X-ray diffraction (PXRD) and variable temperature powder X-ray diffraction (VTPXRD). The crystallization kinetics studied by dynamic DSC heating techniques followed by analysis using the Friedman isoconversion method where values of activation energy (E) and frequency factor (A) were estimated as a function of alpha or extent of conversion (α). The variations in E with α, from 0.05 to 0.9, for the amorphous to form B conversion could indicate the formation of intermediate polymorphs prior to form B. The form B to form A conversion showed a constancy in E on kinetic analysis from α 0.05 to 0.9, which suggested that a constant crystallization mechanism operated during formation of the thermodynamically stable form A.

Keywords: Nifedipine; DSC; Polymorphism; Enantiotropic; Crystallization

1. Introduction

The occurrence of polymorphism for solid-state pharmaceuticals is an important phenomenon since it affects their physico-chemical properties [1]. Changes in drug crystal structure will alter physical behaviour in terms of melting, aqueous solubility and solubilisation rate as well as the chemical properties (light sensitivity, thermal stability). The amorphous form of many drugs may often be more desired for pharmaceutical formulations, especially where solubilisation behaviour is concerned [2]. The complicating factor is that the amorphous form is thermodynamically unstable and will tend to crystallise to a more stable modification. Crystallization may also not be a simple process and it could be that a number of polymorphs may exist simultaneously. Drug crystallization in pharmaceutical formulations could as a consequence lead to varied product quality.

This study will focus on the polymorphic transitions of nifedipine (dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine 3,5-dicarboxylate), a calcium-channel blocker, characterised by a high photosensitivity and low aqueous solubility for its most stable modification [3,4]. Eckert and Müller [5] first reported on the nifedipine polymorphism from supercooled melts. They identified three monotropically related modifications, i.e. modification III, melting point (mp) 134–137 °C, modification II (form B), mp 162–164 °C and the thermodynamically stable modification I (form A), mp 172–172.5 °C. Burger and Koller [6] obtained similar findings that crystallization from the supercooled melt produced modification III first (∼80 °C), mp 133–135 °C, which transformed

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to modification II (form B), mp 161–163 °C and modification I (form A), mp 169–173 °C which crystallized from 110 °C.

Aso et al. [7] reported that amorphous nifedipine obtained from the supercooled melt crystallized to a metastable state (form B) at 90 °C, which converted to the thermodynamically stable form (form A) from 110 °C. Hirayama et al. [8] evaluated amorphous nifedipine crystallization in the presence and absence of 2-hydroxypropyl-β-cyclodextrin and confirmed from powder X-ray diffraction (PXRD) data, that chilled nifedipine heated to 110 °C, crystallized to form B. This form converted to form A (stable modification) at 125 °C, which melted at 171 °C. The presence of 2-hydroxypropyl-β-cyclodextrin retarded the crystallization of amorphous nifedipine to form A and a form B melt was reported at 163 °C. Further evaluation of the crystallization kinetics from the amorphous state was performed using the method of Torfs [9] whereby the Arrhenius parameters were determined using the resolved crystallization exotherm from a single differential scanning calorimeter (DSC) run. The process reportedly followed second-order kinetics and the activation energy were calculated to be 642 kJ mol^{-1}.

Zhou et al. [10] reported that the metastable form II (form B), produced on isothermal crystallization was enantiotropically related to another modification (form C) which formed on cooling form B, below 30 °C. This modification converted back to form B, endothermically, on reheating at 60 °C. Zhou et al. [10] studied the crystallization kinetics for the amorphous nifedipine to form B conversion, using Model-Fitting and Model-Free approaches. The observation of spherulitic growth at the crystallization temperatures from 90 °C were attributed to form B formation. They concluded that an Avrami-Erofeev mechanism (with changing n) operated during the amorphous nifedipine crystallization process. A activation energy (E) of ∼130 kJ mol^{-1} was estimated from model-free isoconversion methods, with E little changed over the alpha (α) range 0.05–0.80. Keymolen et al. [11] reported that amorphous nifedipine crystallization may involve the formation of polymorphs III and IV from liquid–solid and solid–solid transformations, which both converted to metastable form II (form B). They attributed spherulitic growth and the formation of needle-like structures observed by hot stage microscopy (HSM) between 85 °C and 105 °C, to polymorphs III and IV, respectively. These conclusions were based on DSC analysis and the changes for the storage and loss heat capacity, obtained from modulated temperature differential scanning calorimetry (MTDSC) analysis. This was in contrast to the findings of Burger and Koller [6] who reported that modification III, first produced from the melt transformed into spherulites, form II (form B). The work of Burger [6] and Keymolen [11] does correspond in terms of the reasoning that crystallization from the melt produced other polymorphs prior to form B and stand in contrast to the relative constancy in activation energy reported by Zhou et al. [10], over the initial stages of melt crystallization.

This paper will consider a range of thermal analysis techniques, including variable temperature X-ray diffraction (VTPXRD), to explain some reported discrepancies for amorphous nifedipine crystallization as well as investigate the existence of enantiotropically related modifications.

2. Experimental

2.1. Materials

Nifedipine was purchased from Sigma–Aldrich, and used without further purification. Solvents for HPLC and mobile phase preparation included methanol (HPLC grade), obtained from Riedel-deHaën, double distilled water and disodium hydrogen phosphate, obtained from Saarchem.

2.2. DSC and TG analysis to study nifedipine thermal behaviour

Differential scanning calorimetry (DSC) was performed on a TA Instruments Du Pont 910 Standard DSC module, connected to a TA 2000 Thermal Analyzer. For thermogravimetry (TG) a TA Instruments TGA 2050 module, connected to a TA 2000 Thermal Analyzer, was used. High purity nitrogen, at a flow rate of 65 mL min^{-1}, was used as the purge gas. Samples for DSC were encapsulated using aluminium pans and lids. Samples for TG were placed on a platinum pan and suspended in the heating chamber. Both instruments were calibrated using indium and zinc standards.

2.3. MTDSC analysis to study heat flow changes (reversible and non-reversible) during amorphous nifedipine crystallization

The analysis was performed on a DSC Q 100 TA instrument with setting to modulated temperature DSC mode. The first scan raised the temperature to 190 °C, followed by cooling at 100 °C min^{-1} to 35 °C. The rescan was identical to the first. The modulation during the heating cycles included the oscillation amplitude of 2 °C and oscillation period of 60 s, isothermal for 0.06 min with the average heating rate at 5 °C min^{-1}.

2.4. HSM for visual identification of polymorphs during nifedipine polymorphic conversions

Solid–solid transformations were studied using a Nikon Eclipse E400 (Nikon, Japan) thermomicroscope, with a Methatherm 1200d heating unit (HSM) and a Nikon Coolpix 5400 digital camera (Nikon, Japan). Samples were placed on an object plate, covered with a cover plate and observed under the thermomicroscope at a temperature range from 25 °C to 180 °C.

2.5. DSC/PXRD combined technique for characterization of nifedipine polymorphs

Nifedipine was loaded in an empty DSC pan to maximum capacity (∼30 mg). This was followed by DSC heating beyond the normal crystalline melt (∼200 °C), sample removal and cooling at ambient temperature. The sample (an amorphous glass) was pulverised prior to XRD analysis. In a further experiment, again ensuring DSC pan filled to maximum capacity amorphous nifedipine was heated to 114 °C where after the sample

was removed and cooled at ambient temperature (~25 °C). The sample was pulverised prior to XRD analysis. The X-ray powder diffraction data for nifedipine crystal forms was obtained using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; with a 0.020° step and 2 s step time.

2.6. VTPXRD for studying polymorphic conversions on heating nifedipine from the supercooled melt

XRD analysis at 25 °C and at increased temperatures, variable temperature X-ray powder diffraction (VTXRPD), was performed on the crystals. The scanning speed was 2 ° min^{-1} (step size, 0.025°; step time, 1.0 s). For the VTPXRD an Anton Paar TTK 450, low temperature camera (Anton Paar, Austria), was attached to the Bruker D8 Advance diffractometer (Bruker, Germany). An aluminium sample holder was used, and approximately 200 mg of powdered sample was loaded into the sample holder. Amorphous nifedipine, prepared by the melting method, was subjected to VTPXRD analysis at 10 °C intervals from 70 °C to 140 °C. In another experiment was amorphous nifedipine heated on a hot plate to approximately ~114 °C. Visual observation showed that a crystallization process had occurred up to that temperature. The sample was removed, pulverised and subjected to VTPXRD with temperature increase ranging from 25 °C to 120 °C.

2.7. Evaluation of the crystallization kinetics for the amorphous to form B and form B to stable form A conversions

2.7.1. Amorphous to form B crystallization

Accurately weighed nifedipine samples (approximately 3 mg) on lightweight aluminium foil were heated on a hot plate to 190 °C. The melted samples were removed after 1 min to ambient temperature conditions with the now solidified glassy samples reweighed in DSC alumina pans prior to analysis. The pans containing amorphous nifedipine were not sealed with an aluminium cover lid when DSC heated in a nitrogen atmosphere at rates that varied from 0.5 °C min^{-1} to 4.5 °C min^{-1}, from 30 °C to 190 °C. The heat rates employed were slow enough to ensure good resolution for the amorphous to form B and form B to form A crystallization processes.

2.7.2. Form B to form A crystallization

This process was studied by heating amorphous nifedipine (as prepared in Section 2.7.1) in an open DSC pan at 10 °C min^{-1} to ~111 °C. The sample was removed, allowed to cool to ambient temperature (~25 °C) and reweighed. The temperature of ~111 °C was sufficient since HSM showed all amorphous nifedipine had crystallized to form B, while no form B to form A conversion was observable. Samples of approximately 3 mg were DSC heated from 30 °C to 190 °C, at rates that varied from 0.5 °C min^{-1} to 4.5 °C min^{-1}.

2.7.3. Friedman isoconversion method for evaluation of crystallization processes

The crystallization exotherms obtained from the dynamic DSC heating experiments (Section 2.8) were evaluated using the Friedman isoconversion method [12]. The procedure permitted the estimation of activation energy (E) as a function of the extent of reaction (α). The DSC crystallization exotherms were analysed using Universal Analysis software, provided by Setpoint Instruments. The crystallization exotherm for the conversion of amorphous nifedipine to form B, was integrated using a sigmoidal baseline. A linear baseline was used for the form B to form A exotherm. Alpha (α) temperature (T) curves were constructed by consideration of the running integral over the crystallization temperatures. The value of the integral (J g^{-1}) at a specified temperature divided by the total integral for the crystallization exotherm, provided an estimate of α at that specified temperature. The α–T data obtained at various heating rates (β) for the crystallization processes were used to calculate $d\alpha/dT$ values for α values from 0.05 to 0.9. Plots of $\ln[(d\alpha/dT)\beta]$ versus $1/T$ (K^{-1}) for each α value were constructed with slopes = $-E/R$ and the intercepts = $\ln[Af(\alpha)]$. The value of frequency factor (A) was estimated by extrapolation of a plot of the intercept against α_i to $\alpha_i = 0$.

2.8. HPLC analysis for possible decomposition products during thermal treatment

The nifedipine samples subjected to thermal treatment by DSC techniques, was dissolved in a minimum amount of methanol (5–10 mL) and diluted to 25 mL with a 50% methanol/water (v/v) solution. The calibration solutions were prepared using aliquots from the working solution (100 μg mL^{-1}). The concentrations of the nifedipine standard solutions were 16 μg mL^{-1}, 12 μg mL^{-1}, 10 μg mL^{-1}, 8 μg mL^{-1}, 6 μg mL^{-1}, 4 μg mL^{-1}, 2 μg mL^{-1} and 1 μg mL^{-1}.

The prepared sample and calibration solutions were filtered through 0.45 μm prior to HPLC analysis. A 25 μL aliquot from the filtered solutions was injected into a Waters HPLC system comprising a 600 E multisolvent delivery system connected to a 996 photodiode detector. Data analysis was performed on Millennium software Version 1.0. The chromatographic conditions employed included a reversed phase Nucleosil-C18-bondapack column (5 μm particle size), 150 mm × 3.9 mm. The mobile phase consisted of a 0.0100 M disodium hydrogen phosphate/methanol solution (45:55 v/v). The pH of the disodium hydrogen phosphate solution was adjusted to 6.1 using 50% phosphoric acid, before mixing with methanol. The flow rate was 1.0 mL min^{-1}, with detection wavelength at 254 nm.

3. Results and discussion

3.1. Thermal behaviour of nifedipine

The DSC curve for crystalline nifedipine (Fig. 1a), heated at 5 °C min^{-1} in nitrogen, showed a sharp melting endotherm in the region of 171 °C, followed by several broad overlapping thermal events (exo and endo) between 200 °C and 450 °C. The

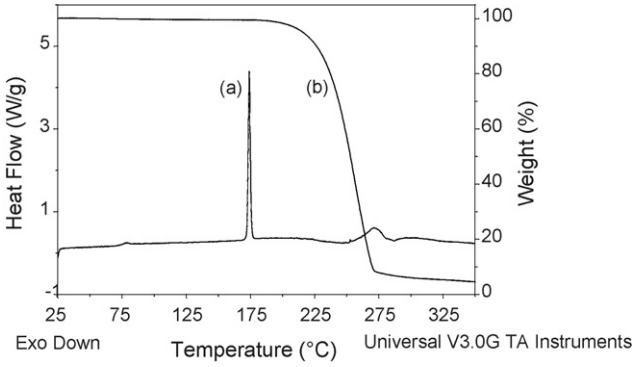

Fig. 1. DSC (a) and TG (b) curves for nifedipine heated at 5 °C min^{-1} in nitrogen.

TG curve (Fig. 1b also at 5 °C min^{-1} in nitrogen) showed an onset of mass loss after the melting point (∼172 °C). The total mass loss was more than 90% by 300 °C, and the smooth nature of the TG curve suggested possible evaporation of the liquid. The mixture of endothermic and exothermic events observed in the DSC curve indicated, however, that decomposition was also involved.

The region above the nifedipine melt was investigated to ascertain the temperature regions for nifedipine loss due to evaporation as well as evaporation accompanied by decomposition. The is important since the investigation of nifedipine polymorphism involved preparation of the amorphous modification by heating above the crystalline melt (171–173 °C) to 190 °C, followed by supercooling at ambient temperatures. The activation energy for nifedipine evaporation was calculated from the TG curve using the method of Dollimore and Lerdkanchanaporn [13]. Dollimore assumed evaporation to be a zero-order process, which implied that the evaporation rate (mg min^{-1} cm^{-2}) is equal to the rate constant (k). The DTG signal divided by the surface area of the pan (0.2827 cm^2) would give k (mg min^{-1} cm^{-2}). An Arrhenius type plot (ln k versus $1/T$) tested for linearity over various temperatures established the temperature limits where evaporation would be the dominating process for nifedipine loss. The plot (Fig. 2) showed the non-linearity at temperatures above 260 °C and indicated that chemical decomposition processes in addition to evaporation became increasingly important. Regression analysis on the linear portion of the curve (200–260 °C) yielded a correlation coefficient (R^2) of 0.998 with activation energy (E) and frequency factor (A) estimated as 138 kJ mol^{-1} (±1) and 1.03×10^{12} s^{-1} (±0.02), respectively.

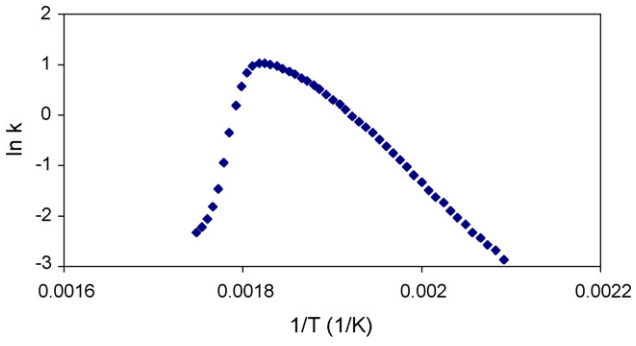

Fig. 2. Arrhenius plot for the zero-order loss (evaporation) of nifedipine over the temperature range 200–300 °C.

Macêdo et al. [14] reported four decomposition stages for nifedipine, from TG data (10 °C min^{-1}) analysed by the tangent method. The kinetic parameters for the first decomposition stage were calculated using the methods of Coats and Redfern [15] and Madhusudanan et al. [16]. Values of 146.1 kJ mol^{-1} and 149.1 kJ mol^{-1} were obtained, respectively. The results from the Arrhenius plot (Fig. 2) suggested that amorphous nifedipine preparation will not be accompanied by any chemical decomposition under the performed experimental conditions.

3.2. Nifedipine polymorphism

3.2.1. DSC

The crystallization behaviour of nifedipine was studied by first preparing the amorphous modification by supercooling of the melt. DSC heating of the amorphous glass (Fig. 3a) at 10 °C min^{-1} showed the characteristic glass transition at ∼46 °C. Crystallization from the amorphous phase was observed from ∼100 °C. The first large crystallization exotherm was followed by a smaller but much broader exotherm over the temperature range ∼120 °C to 152 °C. This exotherm was associated with formation of the thermodynamically stable form A which melted at ∼171 °C. The sample was removed at 190 °C, and cooled at ambient temperature (∼25 °C).

Polymorphic transitions were further investigated by reheating the amorphous glass at 10 °C min^{-1} to117 °C (Fig. 3b). At this temperature the first crystallization exotherm was just completed and nifedipine existed in a metastable form B modification. No major form A formation was expected at this stage of the crystallization process. The sample was removed from the DSC and allowed to cool at ambient temperature conditions. Zhou et al. [10] showed that cooling of form B below 30 °C

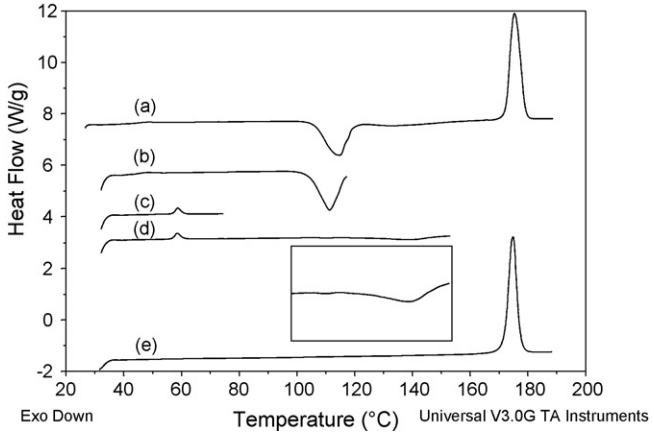

Fig. 3. Crystallization behaviour of nifedipine. (a) Heated at 10 °C min^{-1} to 190 °C, where after sample was cooled at ambient temperature. (b) Sample from (a) reheated at 10 °C min^{-1} to 117 °C where after sample was cooled at ambient temperature. (c) Sample from (b) reheated at 10 °C min^{-1} to 75 °C followed by cooling at ambient temperature. (d) Sample from (c) reheated at 10 °C min^{-1} to 155 °C followed by cooling at ambient temperature. (e) Sample from (d) reheated at 10 °C min^{-1} to 190 °C.

would favour its enantiotropically related modification, form C. Reheat of the sample (Fig. 3c) showed a small endotherm, with onset at 56 °C (~2.4 J g^{-1}) which was assigned to reformation of form B. When the sample was removed at 75 °C, cooled at ambient temperature and reheated at 10 °C min^{-1}(Fig. 3d), this endotherm reappeared which confirmed the enantiotropy between forms C and B. A small exotherm appeared from 120 °C and had occurred to completion when the sample was removed at 155 °C. This process was associated with crystallization to the stable form (form A), as the last reheat (Fig. 3e) showed no endotherm at 56 °C and only the form A melt at ~171 °C. HPLC analysis of the thermally treated sample revealed negligible decomposition of nifedipine (<0.5%). A single nifedipine peak, mean retention time 6.2 min, was obtained in the chromatograms for both sample and calibration solutions.

An amorphous sample (prepared by the fusion method) was subjected to various DSC heat rates. In the first heat stage amorphous nifedipine was prepared by heating untreated nifedipine at 10 °C min^{-1} (Fig. 4a) beyond the normal melt, removed from the DSC and left to cool at ambient temperature. The same sample was again subjected to DSC heating (15 °C min^{-1}) to beyond its normal melt, where after the sample was removed to ambient temperature. Successive heat stages were made progressively faster (Fig. 4b–e) and ranged from 15 °C min^{-1} to 40 °C min^{-1}. The overall mass loss for the sample after the treatment was ~0.7%. HPLC analysis of the thermally treated samples revealed negligible nifedipine decomposition (<0.7%).

Faster heating rates (Fig. 4) caused the amorphous—form B conversion to shift to higher temperature as seen from the upward move of the crystallization exotherm position. The delayed form B to form A crystallization process did not occur to completion at the faster heating rates. Evidence for the incomplete crystallization came from observation of another melting endotherm (~163 °C) preceding the normal melt at ~171 °C. The area of this melting endotherm enlarged with increased heat rate and affirmed the association of the first crystallization exotherm to the formation of another polymorph. The melt was attributed to the metastable modification, classified as form B.

Hirayama et al. [8] reported the enthalpy of fusion of form A as 82 J g^{-1} and that of form B as 69 J g^{-1}. In the present study, the enthalpy of fusion of stable nifedipine (form A) appears to be higher, ~104 J g^{-1}. The enthalpy of fusion of form B was estimated by considering the DSC curves (Fig. 4a–c) obtained at heating rates10 °C min^{-1}, 15 °C min^{-1} and 20 °C min^{-1}. 5.35 mg of nifedipine (Fig. 4a) gave an enthalpy of fusion for the form A melt of ~104 J g^{-1}. The DSC curve for the sample reheated at 15 °C min^{-1} (Fig. 4b), where the area of the endotherm for the form A crystalline melt (~94 J g^{-1}) corresponded to ~90% form A crystalline content. The amount of form B, assuming negligible mass loss due to evaporation, was then ~10% of the sample mass. The area of the form B melting endotherm then provided an estimate of the enthalpy of fusion of form B as ~70 J g^{-1}. The calculation was repeated for the 20 °C min^{-1} (Fig. 4c) experiment with form B enthalpy of fusion estimated as ~71 J g^{-1}.

3.2.2. MTDSC

Nifedipine crystallization from the solidified melt was studied by subjecting the sample to a sinusoidal temperature modulation with 0.06 min isothermal and average heating rate at 5 °C min^{-1} to 190 °C. The first MTDSC run involved heating crystalline nifedipine (form A). No discontinuities were observed in the reversible, total and non-reversible heat flow signals (Fig. 5a inset bottom curve, b and d, respectively) up to the melt of the thermodynamically stable form A at ~170 °C. The melted nifedipine sample was supercooled at 100 °C min^{-1} to 35 °C. The supercooled melt was subjected to the same temperature modulation during the reheat. Reheating of the solidified glass to the crystallization temperatures showed amorphous nifedipine to crystallise (from ~92 °C) irreversibly from the melt as observed from the similarities in the total and non-reversible heat flow curves (Fig. 5c and e). The conversion processes from amorphous (considered a high free energy state)

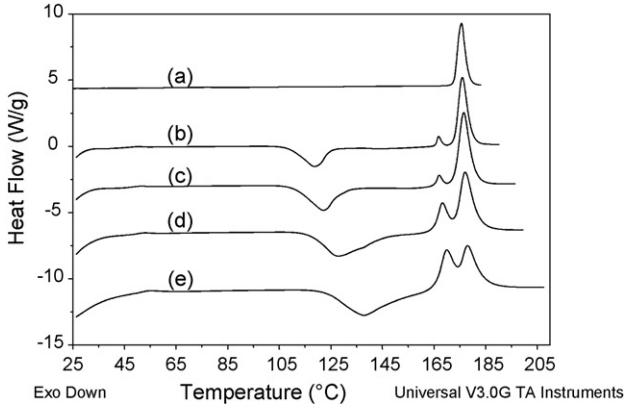

Fig. 4. Effect of the heating rate on crystallization behaviour of amorphous nifedipine for: (a) sample heated at 10 °C min^{-1} to 190 °C, followed by cooling at ambient temperature. (b) Sample from (a) reheated at 15 °C min^{-1} to 190 °C, followed by cooling at ambient temperature. (c) Sample from (b) reheated at 20 °C min^{-1} to 190 °C, followed by cooling at ambient temperature. (d) Sample from (c) reheated at 30 °C min^{-1} to 190 °C, followed by cooling at ambient temperature. Sample from (d) reheated at 40 °C min^{-1} to 190 °C.

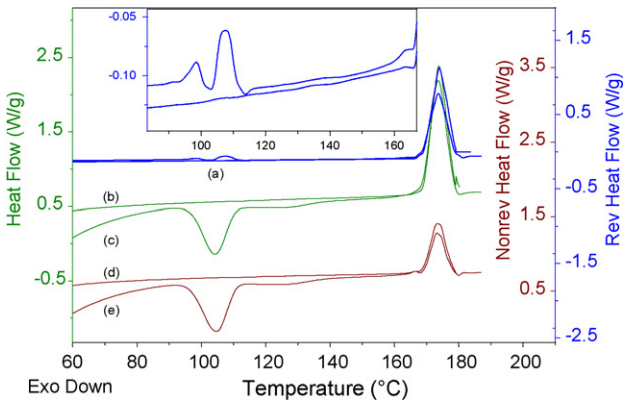

Fig. 5. MTDSC for nifedipine heated to 190 °C; cooled 100 °C min^{-1} to 35 °C and reheated to 190 °C. The average heating rate during heat stages was 5 °C min^{-1}. Heat flow curves include (a) reversing (inset view) signals for crystalline (bottom) and amorphous (top); total heat flow for (b) crystalline and (c) amorphous; non-reversible heat flow for (d) crystalline and (e) amorphous nifedipine.

to metastable (form B) and lastly to thermodynamically stable form A occurred irreversibly down the free energy ladder. The reversing heat flow signal (Fig. 5a inset top curve) showed an irregular shaped curve during the crystallization from the amorphous phase. The general reversing signal baseline decrease up to form B formation at ∼112 °C suggested that the amorphous to form B conversion was accompanied by a decrease specific heat. Keymolen et al. [11] reported that amorphous nifedipine crystallization, studied at a slower underlying heat rate (0.5 °C min^{-1}) for iso/scan and heat/cool modulations, was accompanied by a stepwise downward shift in the reversing signal, attributed to liquid–solid and solid–solid recrystallization processes. The irregular shaped reversing signal obtained at the faster underlying heating rate (5 °C min^{-1}) in this study could not provide any

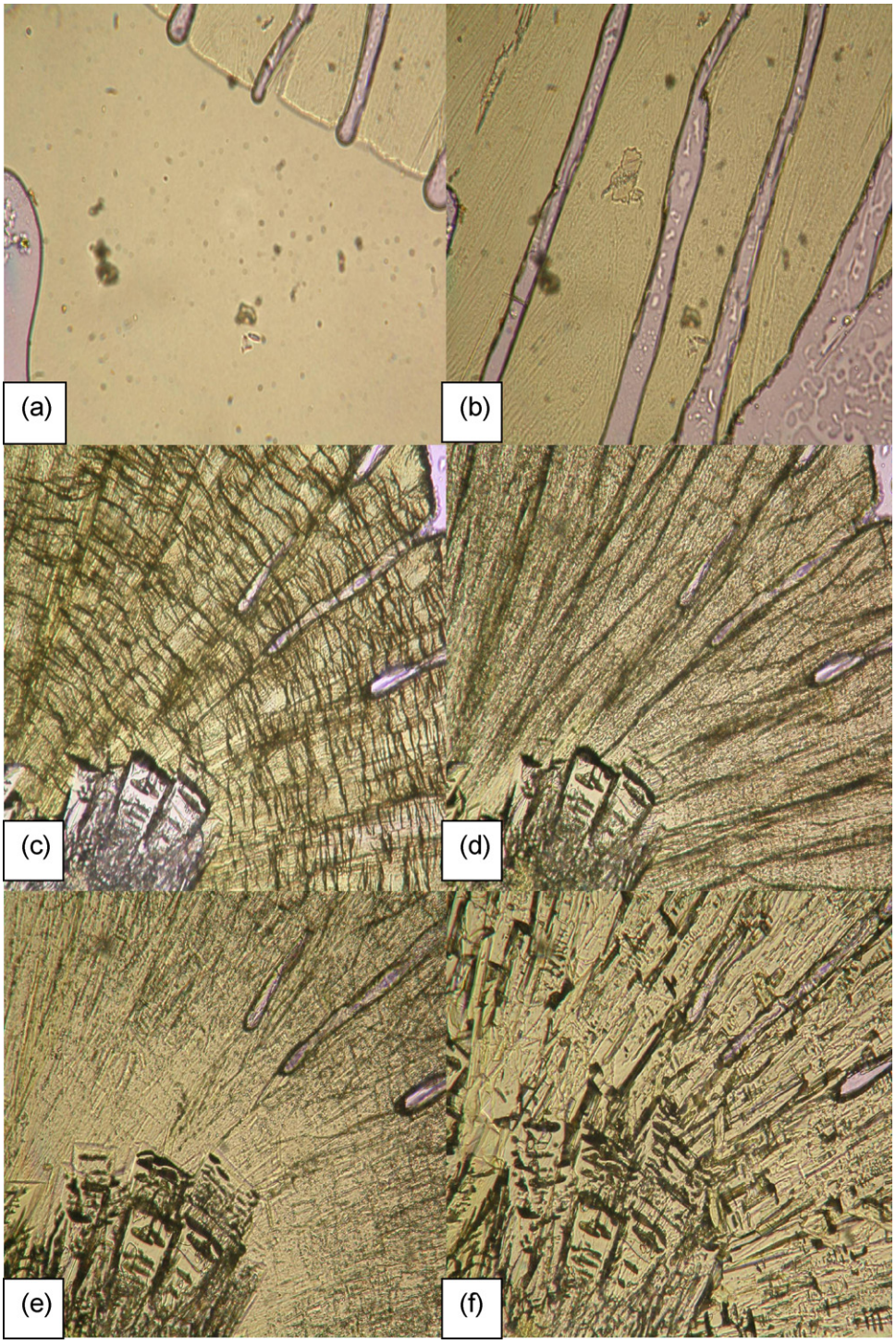

Fig. 6. Hot stage microscopy using Nikon Eclipse E400 (Nikon, Japan) thermomicroscope showed crystallization of amorphous nifedipine at (a) 97 °C and (b) 101 °C to the metastable modification (form B); form C at (c) ∼25 °C converted (from 58 °C) to form B shown here at (d) 75 °C; onset (e) of form B to form A crystallization at 115 °C and (f) form A at 140 °C.

information in terms of particular liquid–solid and solid–solid transformations but the absence of reversing signal curve irregularities during form B to form A conversion over the temperature range ∼112 °C to ∼145 °C, suggested that melt crystallization may include polymorphs intermediate between amorphous and form B. The complexity of the crystallization exotherms (amorphous to form B and form B to A) was investigated by kinetic means in Section 3.3.

3.2.3. Hot stage microscopy (HSM)

Visual observations of the nifedipine polymorphic transitions were investigated using hot stage microscopy (HSM). About 3 mg nifedipine was placed on a microscope slide with a cover plate to eliminate any visual problems created by multidimensional crystal growth. The first heating procedure raised the temperature of the hot stage from ambient to 180 °C. The melted sample was removed and allowed to cool at ambient temperature. Nifedipine was now an amorphous glass. The temperature of the amorphous glass was then raised in order to visualise crystallization to the metastable (form B) modification. Spherulitic crystal growth from the melt was observed from 97 °C which corresponded approximately with the observed crystallization exotherm from Fig. 3a. Fig. 6a and b showed crystal growth spreading through the amorphous phase at temperatures 97 °C and 101 °C, respectively.

The sample (form B) was removed at 110 °C from the hot stage and allowed to cool to ambient temperature. The cooling process facilitated the formation of form C. The form C modification (Fig. 6c) exhibited crystal packing which appeared to be aligned parallel with the outward growth direction starting from the edges of an irregular shaped nucleus spreading outwards in zones of crystal packing away from the central nucleus. Interspersed among these zones of crystal packing was dark coloured crystal growths aligned perpendicular to the outward growth direction. Hot stage microscopy showed these structures to losen and realign parallel with the zonal crystal growths from 58 °C. The morphological changes was observed over a narrow temperature range (58–63 °C) and coincided with the reported enantiotropy between modifications C and B from DSC (Fig. 3c). Fig. 6d represents the sample at 75 °C, where nifedipine existed as the metastable form B modification.

Further heating of form B showed gradual structural changes (Fig. 6e) observable over the entire surface, from 115 °C. The coarse crystal surface gradually smoothened, became platy with dark structures of crystal packing again appearing randomly on the surface. The dark structures exhibited no particular orientation, but their formation was associated with a smoothening of the crystal surface and grooves developing parallel to the length of the original spherulitic form. These changes corresponded with crystallization to the stable form (A) and coincided with the second crystallization exotherm from the DSC experiment (Fig. 3d). No further changes were observed after 130 °C and indicated that crystallization (Fig. 6f) was complete.

3.2.4. DSC/PXRD

PXRD studies were performed so as to validate the conclusions from DSC and HSM. Untreated nifedipine (as obtained from Sigma–Aldrich) was assumed to exist in its most stable crystalline form and classified as form A. PXRD analysis confirmed the crystalline nature of nifedipine (Fig. 7 top). Characteristic intense diffraction peaks (>450 counts) were noted at 8.2°, 10.5°, 11.7°, 16.2°, 24.7° and 26° for 2θ. A halo pattern (Fig. 7 middle) characteristic of the amorphous form for nifedipine was obtained after supercooling of the nifedipine melt. The diffraction pattern attributed to the third modification, classified as form C, was obtained using a combined DSC–PXRD technique. Amorphous nifedipine was DSC heated at 10 °C min^{-1} to just beyond the first crystallization event (∼114 °C) to ensure that all nifedipine converted to the form B modification. It was expected that sample removal to ambient temperature conditions should facilitate the phase transition to form C. The diffraction pattern (Fig. 7 bottom) showed more peaks which was distinctly different from form A. This confirmed the existence of another crystalline form for nifedipine. Some prominent peaks for 2θ were observed at 7.4°, 9.2°, 11.2°, 12.4°, 12.8°, 15.7°, 16.9°, 24.2° and 26.3°.

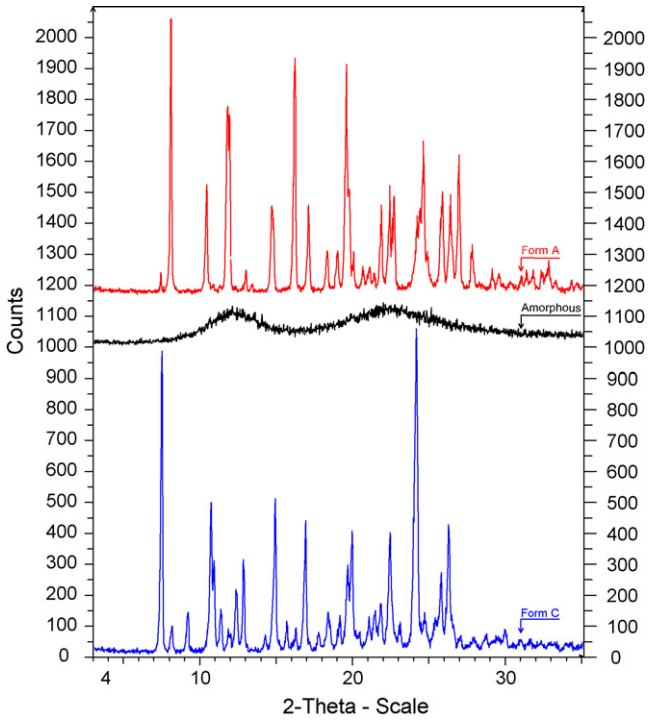

Fig. 7. Powder X-ray diffractograms for three modifications of nifedipine. From top: nifedipine in form A state as obtained from Sigma–Aldrich, middle: amorphous nifedipine prepared by DSC heating of form A at 10 °C min^{-1} to 200 °C followed by cooling in at ambient temperature and below: form C prepared by DSC heating of amorphous nifedipine at 10 °C min^{-1} to 114 °C followed by cooling at ambient temperature.

3.2.5. VTPXRD

3.2.5.1. Amorphous nifedipine crystallization to stable form. This analysis was performed to evaluate diffraction pattern changes during nifedipine crystallization from the amorphous to the stable form A modification. The DSC experiments showed that amorphous nifedipine crystallized via a transient metastable form B state which should show some differences in terms of the

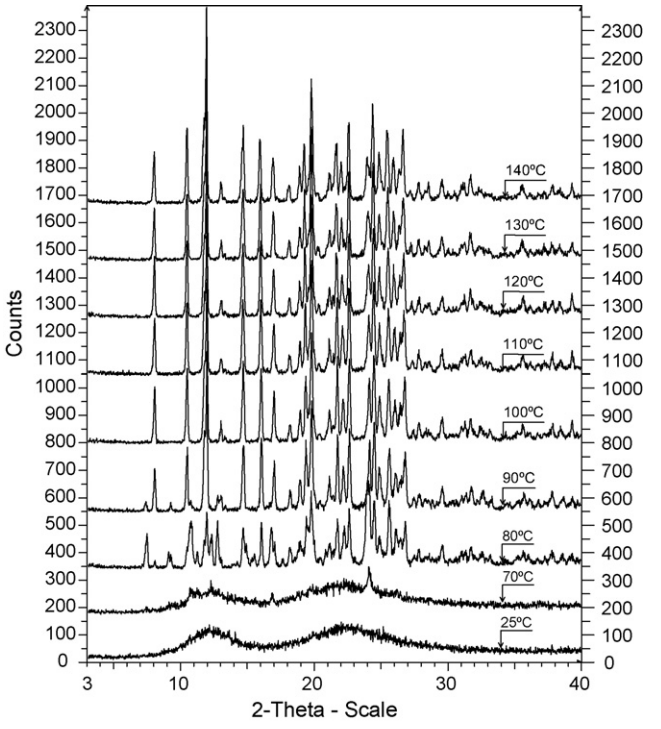

Fig. 8. VTPXRD analysis to show crystallization of amorphous nifedipine heated from 25 °C.

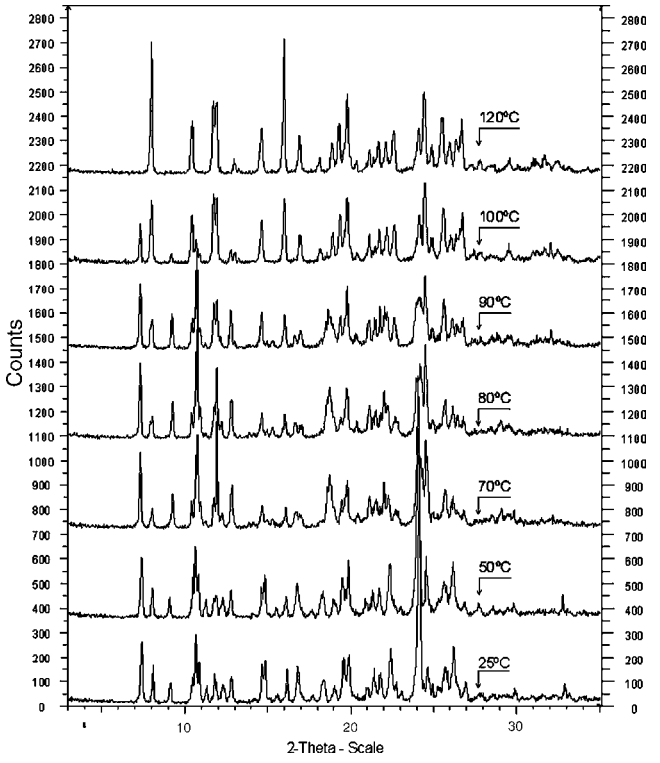

Fig. 9. VTPXRD analysis to show phase transformations from the form C nifedipine modification, heated from 25 °C.

diffraction pattern, relative to the patterns obtained for forms A and C. Pulverised amorphous nifedipine (100 mg) was subjected to XRD analysis at 10 °C intervals from 70 °C to 140 °C. This would enable the observation of possible transformations from the amorphous to the stable crystalline state.

The XRD pattern changes (Fig. 8) indicated an onset of crystallization from 70 °C. At 80 °C, a pattern emerged which showed that crystallization from the amorphous phase occurred via some intermediate phase, assigned to metastable form B. Peaks of form A already emerged at 8.2° but was more evident between 15° and 28°. Although the DSC analysis did not indicate any thermal events at this temperature the observation of crystallization can be attributed to the pulverised state of the introduced sample as well as the long analysis time (25 min) at each temperature. By 100 °C the diffraction pattern for the stable form (form A) was complete with little changes being observed up to 140 °C.

Form C crystallization to stable form A. The form C modification, prepared by heating amorphous nifedipine on hot plate to ∼114 °C and cooling at ambient temperature, was pulverised and subjected to variable temperature/PXRD. Fig. 9 showed an identical pattern at 25 °C to that obtained from the DSC/PXRD (Fig. 7) experiment which confirmed the form C modification. When the temperature was raised to 50 °C and then 70 °C, some changes in the diffraction pattern was observed. It was found that some peaks, attributed to the form C modification completely disappeared, others reduced in intensity with new peaks being observed from 70 °C. Some notable changes for 2θ were observed at 9.2°, 14.8°, 16.8°, 18.4°, 22.2° where most peaks either completely disappeared or sharply reduced in intensity. The most intense peak at 24.2° (singlet) sharply declined in intensity, broadened with neighbouring peak at 24.8° showing steady growth. New peaks at 70 °C occurred at 9.4°, 12.2° and 18.8°. These diffraction pattern changes were associated with the endothermic event, observed at 56 °C, from DSC experiment (Fig. 3c) as well as HSM (Fig. 6) visual observations. It was concluded that the diffraction pattern at 70 °C, configured to the transient metastable form B modification. The conversion to the stable form A was observed from 80 °C. The metastable form B diffraction peaks decreased in intensity and some completely disappeared (7.2°, 9.4°) while the form A peaks increased in intensity from 90 °C.

3.3. Kinetic analysis of the polymorphic transformations

3.3.1. Sample preparation and Friedman isoconversion method

A uniform sample preparation method was followed since the crystallization events are influenced by sample characteristics such as shape and intermolecular attractions within the sample. Amorphous nifedipine, prepared by fusion on a DSC alumina pan and left to cool at ambient temperature conditions, showed spreading behaviour on the pan surface with low uniformity in terms of sample shape and thickness. When amorphous nifedipine was prepared by fusion and ambient temperature cooling on lightweight aluminium foil it resulted in less spreading with greater uniformity in terms of sample shape and thickness. More extensive intermolecular forces operated in the latter samples relative to amorphous modifications prepared on alumina pans as evident from the DSC results (Fig. 10). The crystallization event was shifted to higher temperature and suggested that intermolec-

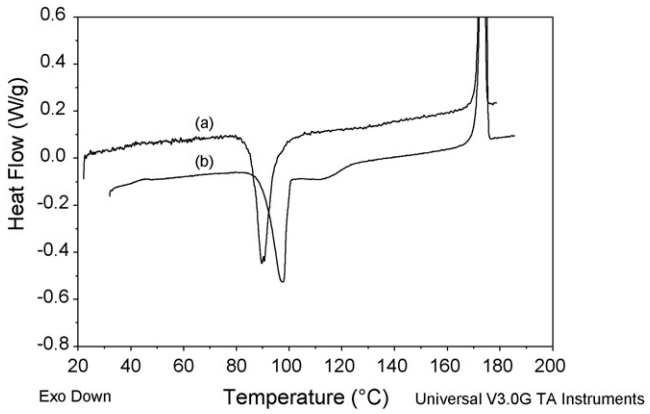

Fig. 10. DSC at 3.0 °C min^{-1} to show the affect of sample preparation on nifedipine crystallization, for amorphous nifedipine prepared by fusion on DSC alumina pan (a) and lightweight aluminium foil (b).

ular attractions first had to be overcome before crystallization could commence. This also reaffirmed the importance of molecular mobility during the crystallization process.

For the kinetic experiments (Section 2.7), the amorphous form of nifedipine was prepared by fusion and cooling at ambient temperature on aluminium foil, prior to DSC analysis. This ensured that each sample was morphologically similar in terms of surface characteristics, internal structure and shape. The form C modification was used as starting material for the kinetic studies on the form B to form A conversion. It was prepared through DSC thermal treatment of amorphous nifedipine, previously prepared on aluminium foil, to ∼111 °C and ambient temperature cooling.

The model-free approach, by Friedman [12], was employed for samples measured at different heating rates. Values of activation energy were estimated as a function of the extent of conversion, alpha (α). Although the value of activation energy cannot indicate the actual crystallization mechanism, its variation with α could provide information on the complexity of the crystallization processes.

3.3.2. Amorphous to form B

The calculations from the non-isothermal data yielded Arrhenius plots that was quite scattered. Values of R^2 varied between 0.94 and 0.97 over the α range 0.05–0.8, but dropped to 0.85 at $\alpha = 0.9$. The activation energy (Table 1, Fig. 12) increased from 109 kJ mol^{-1} (±9) to 164 kJ mol^{-1} (±13) over the α range 0.05–0.6 and then decreased up to $\alpha = 0.9$ to 119 kJ mol^{-1} (±19). The variation in activation energy from the onset of amorphous crystallization implied more complex processes, possibly liquid–solid and solid–solid transformations prior to form B formation. The results was in contrast to the findings of Zhou et al. [10], who reported a constancy in activation energy (∼130 kJ mol^{-1}) over the alpha range 0.05–0.80 from model-free methods. They concluded that amorphous nifedipine crystallized to a metastable form II (form B), according to an Avrami-Erofeev mechanism with changing n. The activation energy variations and the findings from MTDSC (Fig. 5) suggested that form B may not be the only polymorph that nucleate from the melt initially and that form B formation may even be preceded by other polymorphs, possibly modification III [6] or as Keymolen et al. [11] concluded that a number of polymorphs may crystallise from the melt and then be converted to form II (form B).

Table 1
Parameters from Arrhenius plots for the crystallization of amorphous nifedipine for α values 0.05–0.9, calculated using Friedman isoconversion method

α	R^2	Ln[$A(f(\alpha))$]	E (kJ mol^{-1})
0.05	0.958	33.2 (±2.9)	109 (±9)
0.1	0.973	37.8 (±2.6)	121 (±8)
0.2	0.944	43.9 (±4.3)	139 (±13)
0.3	0.945	48.2 (±4.6)	151 (±14)
0.4	0.960	50.3 (±4.1)	157 (±12)
0.5	0.972	53.1 (±3.5)	165 (±11)
0.6	0.960	52.7 (±4.2)	164 (±13)
0.7	0.950	51.3 (±4.6)	160 (±14)
0.8	0.964	50.0 (±3.8)	157 (±11)
0.9	0.848	37.2 (±6.3)	119 (±19)

3.3.3. Form B → form A

The form C modification (Fig. 11) converted endothermically to form B at ∼57 °C. Faster heating rates enlarged this endotherm which made the polymorphic conversion more detectable. At slow heating rates (0.5 °C min^{-1}), form B already started to crystallise from ∼85 °C. Faster heating rates delayed form B crystallization to higher temperature. The heat rates employed was still slow enough to ensure complete conversion of the form B modification to form A, as no form B melt was observed at ∼162 °C to 163 °C. The estimated activation energies for the form B crystallization process was calculated in the α range 0.05–0.9 (Table 2, Fig. 12) and ranged between ∼95 kJ mol^{-1} and 100 kJ mol^{-1}.

Although data scatter was greater for the initial (0.05–0.2) and latter (0.7–0.9) alpha ranges the consistency in the estimated activation energy and intercept was more evident. It was concluded that the mechanism of form B crystallization remained constant for α range 0.05–0.9. The relative constancy in E indicated that the final crystallization exotherm which ultimately

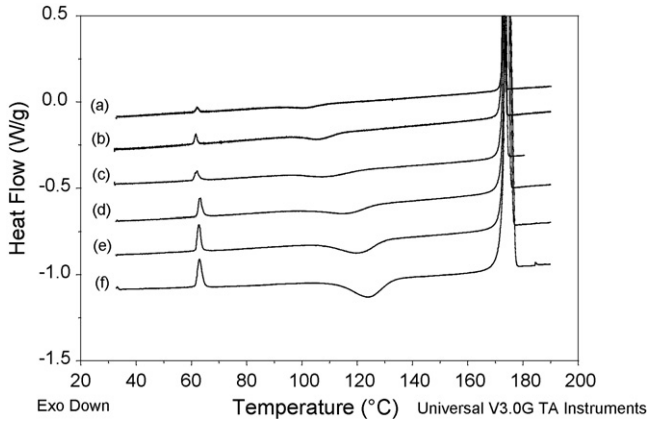

Fig. 11. Nifedipine (form C) heated at various rates to study the form B to form A crystallization process: (a) 0.5 °C min^{-1}, (b) 1.0 °C min^{-1}, (c) 1.5 °C min^{-1}, (d) 2.5 °C min^{-1}, (e) 3.0 °C min^{-1} and (f) 4.0 °C min^{-1}.

Table 2
Parameters from Arrhenius plots for the crystallization of form B to form A for α values 0.05–0.9, calculated using Friedman isoconversion method

α	R^2	Ln[$A(f(\alpha))$]	E (kJ mol^{-1})
0.05	0.938	27.3 (±2.8)	97 (±9)
0.1	0.952	28.1 (±2.5)	98 (±8)
0.2	0.977	27.7 (±1.6)	96 (±5)
0.3	0.971	27.8 (±1.8)	96 (±6)
0.4	0.965	28.7 (±2.1)	99 (±7)
0.5	0.953	27.5 (±2.3)	95 (±7)
0.6	0.953	27.6 (±2.3)	96 (±8)
0.7	0.944	26.9 (±2.5)	94 (±8)
0.8	0.942	28.4 (±2.7)	100 (±9)
0.9	0.926	27.3 (±3.0)	99 (±10)

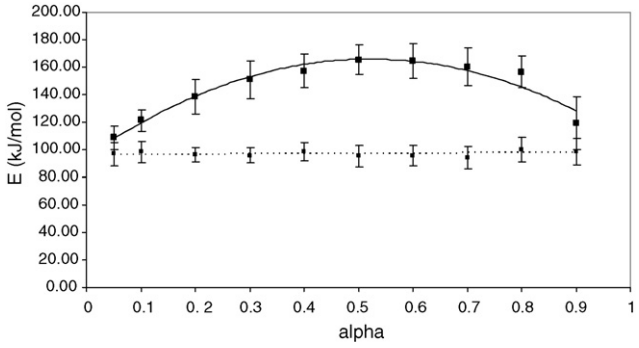

Fig. 12. Plot to show the variation in E (kJ mol^{-1}) as a function of α for the crystallization of amorphous nifedipine to form B (—), and form B to form A (- - -).

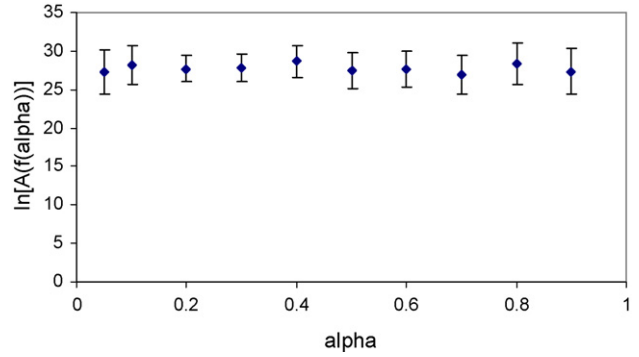

Fig. 13. Plot of the intercept ln[$Af(\alpha)$] against α_i for form B crystallization to form A.

produced form A can only be assigned to one solid–solid transformation process namely form B to A. Extrapolation (Fig. 13) of the intercept to $\alpha = 0$, gave ln(A min) = 27.84 (±0.34).

4. Conclusions

Thermal, microscopic and PXRD analysis confirmed that amorphous nifedipine crystallized to a metastable form B, which on cooling to ambient temperature (∼25 °C) converted to modification C. Polymorph C converted endothermically (∼56 °C to 58 °C) to form B on heating which established the enantiotropic relationship between the two polymorphs. Heating of metastable phase B resulted in crystallization to the stable form A modification, which melted at ∼172 °C to 173 °C. Faster DSC heat rates delayed the form B to form A crystallization process to higher temperature, which resulted in incomplete crystallization. A form B melt was recorded at ∼163 °C, with an estimated enthalpy of fusion of ∼70 J g^{-1} to 71 J g^{-1}. The crystallization of amorphous nifedipine was accompanied by fluctuations in the reversing heat flow signal from MTDSC but also exhibited variations in the estimated activation energies (E) versus α on kinetic analysis. This suggested that a number of polymorphs may nucleate from the melt, which in turn converted to form B. It can be deduced that other liquid to solid as well as solid to solid transformations may be possible prior to form B formation. In summary, the study identified four nifedipine polymorphs (amorphous, form B, form C and form A), with the possibility that during amorphous nifedipine crystallization additional polymorphs may precede form B formation.

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