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RESEARCH PAPER

Effect of Compression on Non-isothermal Crystallization Behaviour of Amorphous Indomethacin

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ABSTRACT

Purpose To evaluate the effect of tablet compression on the physical stability of amorphous indomethacin.

Methods The amorphous indomethacin generated by melt cooling, rapid (5°C/min) or slow (0.2°C/min) cooling, was evaluated by PXRD, mDSC and FTIR analysis. Non-isothermal crystallisation behaviour was assessed using mDSC and any structural changes with compression were monitored by FTIR. Amorphous indomethacin was compressed in a DSC pan using a custom made die cavity-punch setup and further analysed in the primary container to minimize stress due to sample transfer and preparation.

Results Compression of amorphous indomethacin induced and increased the extent of crystallisation upon heating. DSC results revealed that amorphous indomethacin generated by rapid cooling is more prone to compression induced crystallisation than the slowly cooled one. Onset temperature for crystallisation (T_c) of uncompressed slowly and rapidly cooled samples are 121.4 and 124°C and after compression T_c decreased to ca 109 and ca 113°C, respectively. Compression of non-aged samples led to higher extent of crystallisation predominantly into γ-form. Aging followed by compression led to crystallisation of mainly the α-form.

Conclusions Compression affects the physical stability of amorphous indomethacin. Structural changes originated from tablet compression should be duly investigated for the stable amorphous formulation development.

KEY WORDS amorphous · compression · crystallisation · indomethacin · relaxation

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ABBREVIATIONS

DT	dwell time
FTIR (ATR)	Fourier transform infrared spectroscopy (Attenuated total reflectance)
m	minute
mDSC	modulated differential scanning calorimetry
PMMA	poly(methyl methacrylate)
PXRD	powder x-ray diffraction
T_c	onset temperature for crystallisation
T_g	glass transition temperature
TSDC	thermally stimulated depolarization current spectroscopy
ΔC_p	heat capacity change
ΔH_c	heat of crystallization
$\Delta H_f(\alpha)$	melting enthalpy of α-form
$\Delta H_f(\gamma)$	melting enthalpy of γ-form
ΔH_{rec}	enthalpy recovery

INTRODUCTION

High energy amorphous forms can improve kinetic solubility of low to medium molecular weight drugs. This kinetic solubility advantage is often used to improve the bioavailability of hydrophobic drugs having dissolution rate limiting bioavailability. However, their physical instability problem precludes the developability of stable amorphous formulations. The physical instability problem could be adversely affected and aggravated by unit operations, methods of preparation and external stresses such as humidity, high temperature and pressure. The physical stability of amorphous drugs under stress conditions like high temperature as well as humidity has been investigated extensively. However, little has been done to understand the effect of

manufacturing processes like compression induced mechanical and thermal stresses on the physical stability of amorphous drugs (1).

Indomethacin has well characterized α and γ monotropic polymorphs (2). Melt quench amorphous indomethacin has been reported to show non-isothermal crystallisation to preferentially either α or γ-forms depending on the sample preparation method, aging temperature and time (3). Freshly prepared slowly (5°C/min) cooled amorphous indomethacin crystallized dominantly to the γ form whereas the rapidly (liquid nitrogen) cooled one crystallised dominantly to the α form. Moreover, rapidly cooled samples crystallized predominantly to the γ form upon aging for 1 day at 30°C (T_g-16°C) (3,4). In previous studies, the stress imparted by liquid nitrogen and gentle grinding during sample preparation before analysis was shown to impose negligible effect on crystallisation behaviour. In addition Sergey and Ion reported that the effect of liquid nitrogen on crystallization behaviour was not observed at 10°C/min heating rate (5). However Bhugra *et al.* showed that small variation on handling of amorphous material generated by quenching the molten indomethacin using liquid nitrogen resulted in differences in crystallization behavior for samples annealed above T_g (6). Accordingly, they also reported that stressful handling could lead to the formation of both α and γ-forms. Stress conditions such as high temperature ($>T_g$), high humidity (≥95%), high pressure on amorphous slurry favors the formation of the metastable α-form. Generally stress conditions which are associated with increase in molecular mobility can lead preferentially to the α-form (2,7,8).

In the current study we investigated the effect of compression that reflects pharmaceutical tabletting for melt-cooled amorphous indomethacin prepared by slowly and rapidly cooling molten indomethacin. Since most amorphous systems are marketed as tablets and capsules the importance of the effect of compression on physical stability of the final product is ineluctable. Unlike the previous studies, the compression was performed inside DSC aluminum pans using a custom made die cavity-punch setup. The drug in the DSC pan was melt-cooled inside the DSC, followed by compression inside the pan itself and further analysed in the primary container (pan) by DSC, PXRD and FTIR (ATR). All experimental protocols are therefore devoid of external stresses while transferring and preparing (e.g. by slight grinding or using liquid nitrogen) samples for further analysis.

MATERIALS AND METHODS

Material

Indomethacin (98.5–100.5% purity, γ-form) was purchased from Certa n.v. (Braine-l'Alleud, Belgium) and used without further purification.

Methods

Preparation of Amorphous Indomethacin

Amorphous indomethacin was prepared by the melt-cooling technique. About 25 mg of the γ-form of indomethacin powder was properly placed in a standard aluminum DSC pan and melted at 175°C for 5 min (5,9) and subsequently cooled to room temperature at 0.2 and 25°C/min inside the DSC. They will be referred to as slowly and rapidly cooled samples, respectively. Cooling to very low temperature was avoided to minimize the effect of cracking on non-isothermal crystallisation behavior of indomethacin. Preliminary studies revealed that samples that are cooled by liquid nitrogen showed cracking which triggered non-isothermal crystallisation (Fig. 1).

Compression and Aging

Compression at ca 43.7 MPa was applied for 1 s, 2.5 and 5 min dwell time on glassy indomethacin in a DSC pan using a custom made die cavity-punch setup. The compression study was performed using a compression machine with an upper punch driven by compressed air. The custom made die cavity has a bottom diameter equivalent to the external diameter of the pan and a top diameter equivalent to the internal diameter of the pan. The setup was designed to prevent deformation of the pan by protecting its edges from the punch. All melt-cooled amorphous indomethacin was compressed inside the pan and the same was analysed by DSC, PXRD and FTIR (ATR). The samples were analysed as prepared (freshly), after aging for 5 and 10 days in vacuum oven at room temperature (25 ± 0.5°C) before and after compression (n=3). Rapidly cooled samples aged for 10 days in vacuum oven at room temperature were further stored for 83 days in open condition, both compressed and uncompressed, to assess the recrystallisation behaviour.

Purity Assay of Melt-Cooled Indomethacin by HPLC

The purity of both slowly and rapidly cooled indomethacin was assayed in comparison to the starting material. Further the purity of compressed slowly cooled indomethacin was assayed using HPLC. Each purity assay was performed in triplicate and the average values were considered for comparison. The HPLC equipment consisted of a Merck Hitachi pump L7100, an ultraviolet (UV) detector (L7400), an autosampler (L7200), an interface (D7000) and a LiChrospher 60 RP Select-B C-18 (5 μm, 12.5 × 4) (Merck, Darmstadt, Germany) column. Isocratic elution with the mobile phase made up of 68% (*v*/*v*) methanol (HiPerSolv Chromanorm, Belgium) and 32% (*v*/*v*) 50 mM sodium acetate buffer (pH 3.5) was used. The detection wavelength

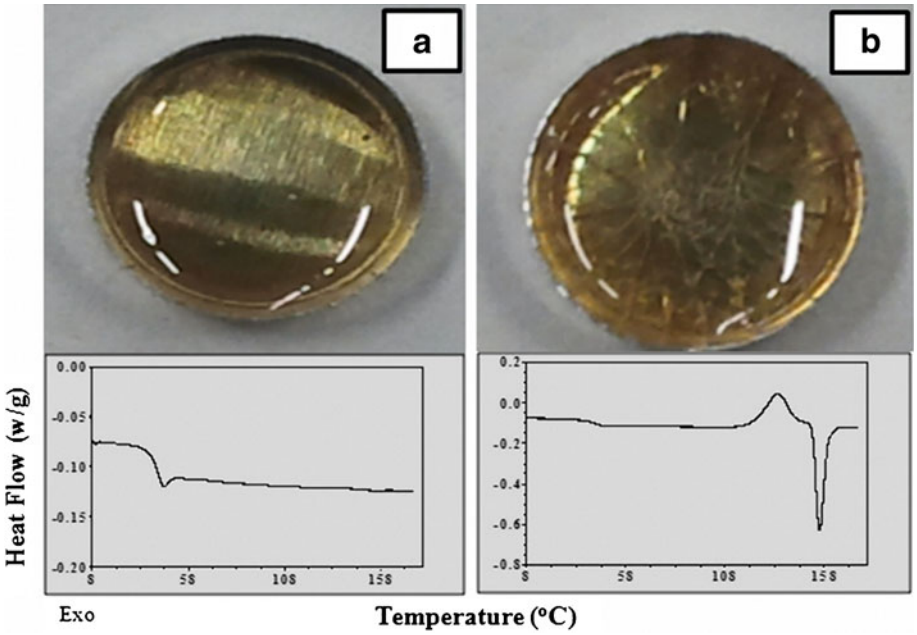

Fig. 1 The sample without cracks prepared by cooling to 25°C at the rate of 25°C/min (**a**) and the cracks created upon quench cooling indomethacin with liquid nitrogen (**b**) with their total heat flow signals as a function of temperature.

and the injection volume were 266 nm and 20 µl, respectively.

Modulated Differential Scanning Calorimetry (mDSC)

The non-isothermal crystallisation behavior of glassy indomethacin was assessed by 2920 mDSC (TA Instruments, Leatherhead, U.K.) equipped with a refrigerated cooling system (RCS) accessory supplied with nitrogen gas (150 mL/min). The DSC cell was purged with helium gas (40 mL/min). The temperature scale was calibrated with octadecane, indium and tin and the enthalpy response was calibrated with standard indium. The heat capacity for mDSC was calibrated by comparing the measured value and literature value at 106.85°C for the sapphire disk using ±0.731°C amplitude every 40 s. The samples were heated from 0°C to 190°C using an underlying heating rate of 5°C/min. The data were analysed using TA Universal Analysis 2000 software (TA Instruments, Leatherhead, U.K.). ΔH_{crys} and T_c were measured by integrating the exotherm event of the non-reversing heat flow signal. The area and the onset temperature for the exothermic signal were taken as ΔH_{crys} and T_c, respectively.

Powder x-Ray Diffraction (PXRD)

The samples in the pan were mounted on a reflection mode PXRD sample holder using two sided tape. They were analysed at room temperature with an automated X'pert PRO diffractometer (PANalytical, Almelo, the Netherlands) with a Cu tube and the generator set at 45KV and 40 mA. The analysis was performed in a continuous scan mode in a range $4° \leq 2\theta \leq 40°$ with 0.0334° step size (1077 number of data acquisition points) and for 600.075 s counting time. The X'pert Data Collector and the X'pert Data Viewer (PANalytical, Almelo, the Netherlands) were used for data collection and analysis, respectively.

FTIR (ATR) Spectroscopy

FTIR spectra were collected using a Nicolet Avatar 370 FTIR spectrometer equipped with a DTGS detector. The untreated and compressed samples in pans were directly mounted over the Smart Orbit single bounce ZnSe ATR crystal by placing the pan upside-down. The spectra were collected using OMNIC spectra software. Thirty two scans were taken for each sample in the spectral range from 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} at room temperature. The spectra were analysed using F. Menges "Spekwin32 - free optical spectroscopy software", Version 1.71.5, 2010, http://www.effemm2.de/spekwin/.

Enthalpy Recovery Study

The enthalpy recovery was measured by mDSC. The corrected enthalpy recovery was estimated by subtracting the effect of frequency (obtained from the cooling curve by using the same modulation parameters) from the value determined on the endotherm of non-reversing heat flow signal near T_g for heating curve (10). This enthalpy recovery was assumed to be equal to the corresponding enthalpy relaxation.

The extent of crystallisation and relaxation between the two paired groups, compressed and uncompressed samples, were statistically analysed by student's t-test.

RESULTS

The purity of rapidly and slowly cooled indomethacin was ca 99% and 95% compared to the starting material, respectively. The purity of slowly cooled indomethacin before and after compression was similar (less than 1% difference) which indicates that compression has no effect on the chemical stability of the amorphous indomethacin.

Amorphous indomethacin was prepared by slowly and rapidly cooling indomethacin melt. In this study much lower cooling rate (0.2°C/min) was used and after 10 days aging it showed halo patterns without Bragg peaks both before and after compression as depicted in Fig. 2. However, compressed samples showed a distinctly different halo pattern with very broad maxima which starts at $2\theta \approx 21°$ and continues till $2\theta \approx 28°$. Moreover, the presence of characteristic peaks at 995 and 1437 cm^{-1} and the absence of crystalline peak at 1189 cm^{-1} in the spectral profile of all amorphous indomethacin as depicted in Fig. 3 ruled out the possibility of any crystallinity. The emergence of a characteristic peak at 1735 cm^{-1} for the non-hydrogen bonded carbonyl group of the carboxylic acid functionality of indomethacin before and after compression confirmed the amorphicity of the systems (11). Besides, the DSC thermograms also showed more or less similar ΔC_p for both slowly and rapidly cooled samples with and without compression (Table I). The difference in ΔC_p upon aging is not statistically significant ($p > 0.05$) which also showed that the systems were amorphous throughout the study period.

Selective Crystallisation

Slowly cooled amorphous indomethacin showed either no or very small recrystallisation and melting to either γ-form or both α and γ-form and similar phenomena were also observed after aging for 5 days. However after 10 days aging at room temperature very small recrystallisation and melting was observed with α-form or both α and γ-form ($n=3$) which apparently showed gradual increase in the dominance of crystallisation of α-form upon aging. Regardless the aging and dwell time the crystallisation in the slowly cooled sample was more pronounced and increased drastically as evidenced by the emergence of a prominent recrystallisation exotherm and an equivalent melting endotherm upon compression (Fig. 4a). Slowly cooled samples also crystallized to α-form and predominantly γ-form after compression. Interestingly, this trend gradually changed upon aging where compressed samples transformed predominantly to α-form (Fig. 5a). After aging for 5 days, compression induced crystallisation of significantly higher proportion of α than γ-form ($p<0.05$), which eventually crystallised to 100% α-form by 10 days ($n=11$ out of 12 crystallized to α-form) for rapidly cooled samples as indicated in Fig. 4b (bottom).

Overall Extent of Crystallisation

Overall, compression prompted high degree of crystallisation as indicated by significantly high heat of crystallisation (ΔH_c) and low onset temperature for crystallisation (T_c) as shown in Fig. 6 and Table I. Onset temperature for crystallisation decreased after 10 days aging for uncompressed slowly and rapidly cooled glassy indomethacin. T_c after 10 days aging is almost similar to prepared-compressed (freshly) samples for rapidly cooled samples. ΔH_c was also gradually increased with aging and it was higher for rapidly than slowly cooled samples. Compression of glassy indomethacin, both slowly and rapidly cooled, initiated crystallisation to the threshold value and remained the same upon aging. Rapidly cooled samples stored for 83 days after compression showed Bragg peaks which apparently indicates partial recrystallisation of glassy indomethacin to γ-form (Fig. 7(B–D)). The uncompressed samples still showed a halo pattern without Bragg peaks which also confirms their amorphous nature (Fig. 7(E)).

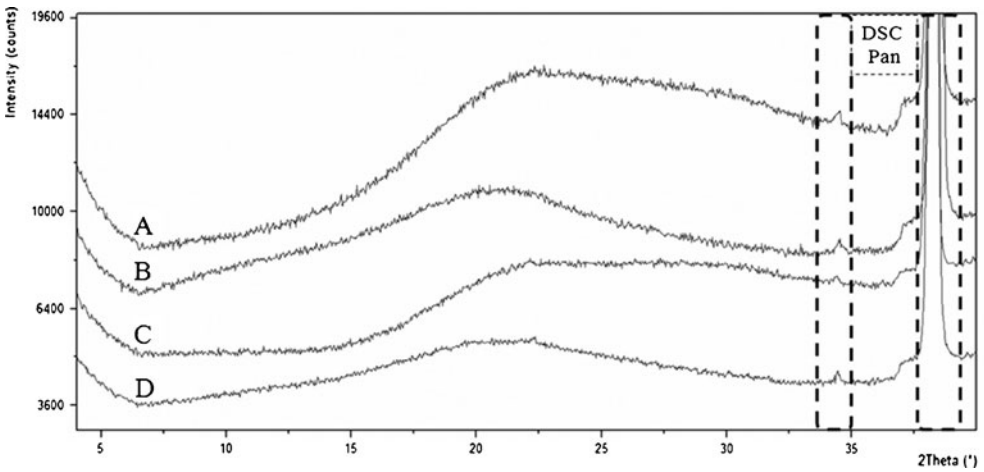

Fig. 2 PXRD pattern of rapidly cooled amorphous indomethacin aged for 10 days (A) compressed at 43.7 MPa for 5 min, (B) uncompressed and slowly cooled ones aged for 10 days (C) compressed at 43.7 MPa for 5 min, (D) uncompressed.

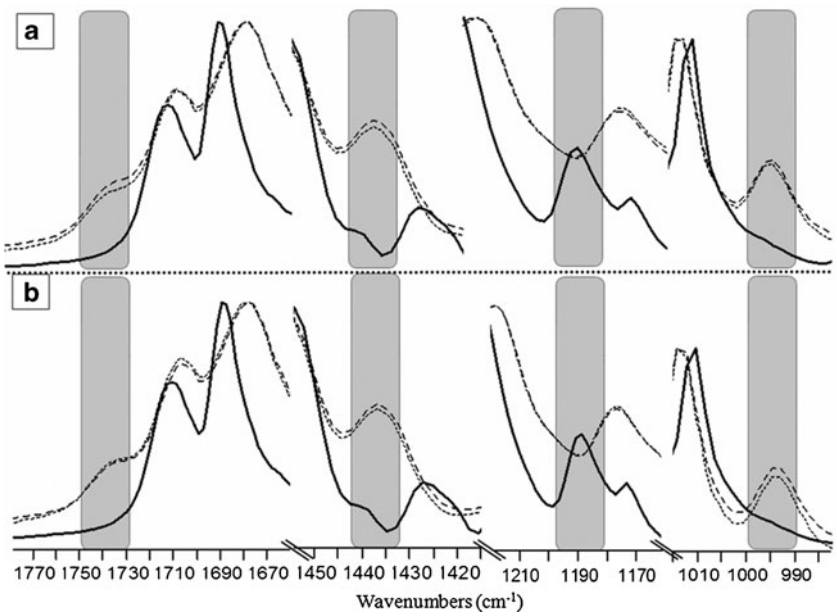

Fig. 3 Partial FTIR spectra (Black solid: γ-indomethacin, Short dashed: amorphous indomethacin after 10 days aging and long dashed: amorphous indomethacin after 10 days aging and compressed at 43.7 Mpa for 5 min) for (**a**) slowly cooled and (**b**) rapidly cooled samples.

Relaxation Behaviour

Slowly cooled samples showed comparatively higher enthalpy recovery than rapidly cooled samples. Compression also showed significantly (statistically) high enthalpy recovery for slowly cooled samples aged for 10 days ($p<0.05$) and 5 days except for 2.5 min dwell time ($p<0.05$). No marked influence of dwell time on relaxation was observed for both samples as shown in Table I for enthalpy relaxation. On the other hand, for rapidly cooled samples there is no clear distinction between relaxation behaviour of compressed and uncompressed samples upon aging, even though compressed samples have high extent of crystallisation and even lower onset temperature for crystallisation than uncompressed sample regardless of aging time.

DISCUSSION

There is a limited understanding on the effect of compression on glassy systems of small organic molecules due to the difficulty of measuring molecular mobility during deformation (12). Generally it has been accepted that deformation enhances molecular mobility that ultimately leads to physical instability problems such as phase separation (13) and crystallisation (14). Compression of stable glassy polymers like poly(methyl methacrylate) (PMMA) nullifies the effect of aging by increasing the energy state of aged glassy system, thus refreshing it back to the energy state of fresh sample, ultimately leading to deformation induced molecular mobility (15,16). Imamura *et al.* also showed that relaxation of sugar matrixes was accelerated to

Table I Enthalpy Recovery (ΔH_{rec}), Onset Temperature for Crystallisation (T_c), Heat Capacity Change at T_g (ΔC_p) as a Function of Aging Time and Compression for Slowly and Rapidly Cooled Samples Determine by DSC (*m* minute, *DT* dwell time). $n=3$; ± sd

		Slowly cooled			Rapidly cooled		
Aging time	Sample	ΔH_{rec} (J/g)	T_c (°C)	ΔC_p (J/(g·°C)	ΔH_{rec} (J/g)	T_c (°C)	ΔC_p (J/(g·°C)
Fresh	Uncompressed	3.6 ± 0.9	121.4	0.36 ± 0.02	1.5 ± 0.7	124.6 ± 8.9	0.36 ± 0.00
	43.7 MPa 1 s DT	3.0 ± 0.2	109.0 ± 0.3	0.34 ± 0.00	1.6 ± 0.5	114.1 ± 1.1	0.35 ± 0.01
	43.7 MPa 2.5 m DT	3.3 ± 0.5	108.3 ± 0.9	0.36 ± 0.02	1.1 ± 0.6	113.1 ± 2.3	0.34 ± 0.01
	43.7 MPa 5 m DT	3.1 ± 0.2	109.1 ± 0.8	0.35 ± 0.02	1.4 ± 0.4	112.7 ± 1.4	0.37 ± 0.02
5 days	Uncompressed	5.4 ± 0.2	123.3	0.39 ± 0.00	5.4 ± 0.2	123.0 ± 1.7	0.38 ± 0.01
	43.7 MPa 1 s DT	6.5 ± 0.1	111.1 ± 2.5	0.38 ± 0.02	5.3 ± 0.3	108.7 ± 3.8	0.34 ± 0.05
	43.7 MPa 2.5 m DT	5.8 ± 0.9	109.1 ± 0.5	0.34 ± 0.05	5.7 ± 0.2	113.2 ± 5.3	0.38 ± 0.02
	43.7 MPa 5 m DT	6.1 ± 0.3	109.4 ± 0.1	0.36 ± 0.02	5.3 ± 0.4	109.9 ± 4.5	0.29 ± 0.01
10 days	Uncompressed	6.0 ± 0.2	115.9 ± 1.7	0.37 ± 0.00	6.1 ± 0.1	118.3 ± 2.6	0.43 ± 0.06
	43.7 MPa 1 s DT	6.8 ± 0.1	109.9 ± 1.5	0.36 ± 0.01	6.1 ± 0.4	116.6 ± 0.8	0.37 ± 0.04
	43.7 MPa 2.5 m DT	7.9 ± 0.6	108.1 ± 0.1	0.35 ± 0.01	5.5 ± 0.4	114.0 ± 4.8	0.35 ± 0.02
	43.7 MPa 5 m DT	7.7 ± 0.5	108.9 ± 0.2	0.35 ± 0.02	5.6 ± 0.3	110.6 ± 5.4	0.33 ± 0.01

the level of a non-porous amorphous sugar matrix after compression (17).

Indomethacin was selected in this study as a model drug due to its good glass forming ability (18). Fukuoka *et al.* were able to generate amorphous indomethacin at very low cooling rate down to 0.67°C/min from melted indomethacin (19). Much lower cooling rate (0.2°C/min) was used in this study and after 10 days aging it showed a halo pattern without Bragg peaks and absence of crystallinity in FTIR (11). However, this cooling rate is much smaller than 1.2°C/min, the minimum cooling rate suggested by Karmwar *et al.* to generate amorphous indomethacin (20). The halo pattern for uncompressed ($2\theta \approx 21°$) differs from that of compressed samples (broader pattern) for different dwell time for both slowly and rapidly cooled samples. This apparently indicates differences in molecular arrangements between them.

Due to their monotropic nature (α and γ-forms) their inter-conversion in solid state is less likely (2). Freshly prepared indomethacin usually crystallized to both polymorphic forms where as it gradually crystallized to a single polymorphic form upon aging (3). Sergey and Ion described that physical aging of amorphous indomethacin is associated with density equalizing and densification. Apparently, freshly prepared glasses have comparatively higher structural heterogeneity and comprises of areas with different local densities. Stimulated by local density gradients, translational diffusion and hydrodynamic flow above T_g and rotational diffusion below T_g act as the driving forces for density equalizing and densification. Relaxation of density fluctuation is apparently fast above T_g due to intense driving forces. This ultimately leads to structurally homogeneous systems with uniform molecular mobility (5,21).

As shown in Fig. 5a for slowly cooled amorphous indomethacin, the ratio of the melting enthalpy of γ and α-form was higher for freshly prepared-compressed samples ($1 < \Delta H_f(\gamma)/\Delta H_f(\alpha) \leq 2.04$) and it gradually decreases for aged and compressed samples. A similar trend was also observed for rapidly cooled samples as depicted in Fig. 5b (10 days aging: $\Delta H_f(\gamma)/\Delta H_f(\alpha)=0$ for $n=11$ out of 12) and uncompressed aged samples also noticeably resulted in crystallisation to nearly a single polymorphic form. This phenomenon was also clearer for rapidly cooled glassy indomethacin aged for 10 days hence it crystallized to a single polymorphic form as indicated in Fig. 4b (A). As expected freshly prepared samples have heterogeneous density and the molecular mobility in local domains with different densities can increase upon compression (5). Recrystallization is a kinetic phenomenon hence it is aging time and heating rate dependent. Relaxation of density fluctuation is faster above T_g due to high molecular mobility and then non-isothermal crystallisation with slow heating rate might give sufficient time lag to facilitate relaxation of density fluctuation in supercooled melt region due to translation diffusion and hydrodynamic flow. It is well established that indomethacin aged above T_g crystallized preferentially to the α-form (3). It seems the heating rate was not slow enough to form a homogenous system above T_g for freshly prepared amorphous indomethacin due their relative high heterogeneity. Compression simply increases the mobility without affecting the heterogeneity of the system as indicated by pronounced crystallisation to both polymorphic forms which is similar to the crystallisation trend of uncompressed samples regarding polymorphic form preference.

After five days aging, compression induced crystallisation of a significantly higher proportion of α than γ-form ($p < 0.05$) ($0.12 \leq \Delta H_f(\gamma)/\Delta H_f(\alpha) \leq 0.28$). Compression of amorphous indomethacin aged for 10 days lead to crystallisation of nearly a single form: α-form especially for rapidly cooled ($n=11$ out of 12 crystallized to α-form). Due to aging the system undergoes densification and density equalization. Hence these can achieve homogenization of glassy indomethacin which can lead to the formation of a single polymorphic form. This relaxation of density fluctuation above T_g is a more important factor for heterogeneous (unaged) than homogenous (aged) amorphous indomethacin in terms of density equalization due to high density of fluctuation of the former. This degree of heterogeneity governs the preference of the polymorphic form formed during heating. Earlier studies apparently showed that aged amorphous indomethacin crystallized to thermodynamically stable γ-form at 10°C/min heating rate (3). On the contrary, this compression induced crystallisation behaviour showed that amorphous indomethacin crystallizes preferentially to the α-form upon aging. Homogenous glasses crystallized to a single polymorphic form regardless of the heating rate. But slow heating rate (5°C/min) could give sufficient lag time above T_g for preferential crystallisation to the α-form as depicted in Fig. 4b. Compression simply increases molecular mobility in aging induced homogenous systems and its effect on density fluctuation is not overt even for 5 min dwell time as confirmed by a single melting transition for rapidly cooled sample after 10 days aging at room temperature. The transition from γ-form dominant to α-form dominant crystallisation was much faster for rapidly cooled samples as indicated by the very steep decrease in slope (Fig. 5) and comparatively very low γ to α melting enthalpy ratio ($\Delta H_f(\gamma)/\Delta H_f(\alpha) \sim 0$) for rapidly cooled samples aged for10 days before and after compression. This could be due to relatively high stability of the residual heterogeneous domains in slowly cooled samples.

Overall compression increases the degree of crystallisation as supported by significantly high ΔH_c (Fig. 6) and low crystallisation induction temperature, T_c (Table I). Broadband dielectric relaxation study on amorphous indomethacin at ambient and elevated pressure showed that the fragility of indomethacin decreases with increasing pressure

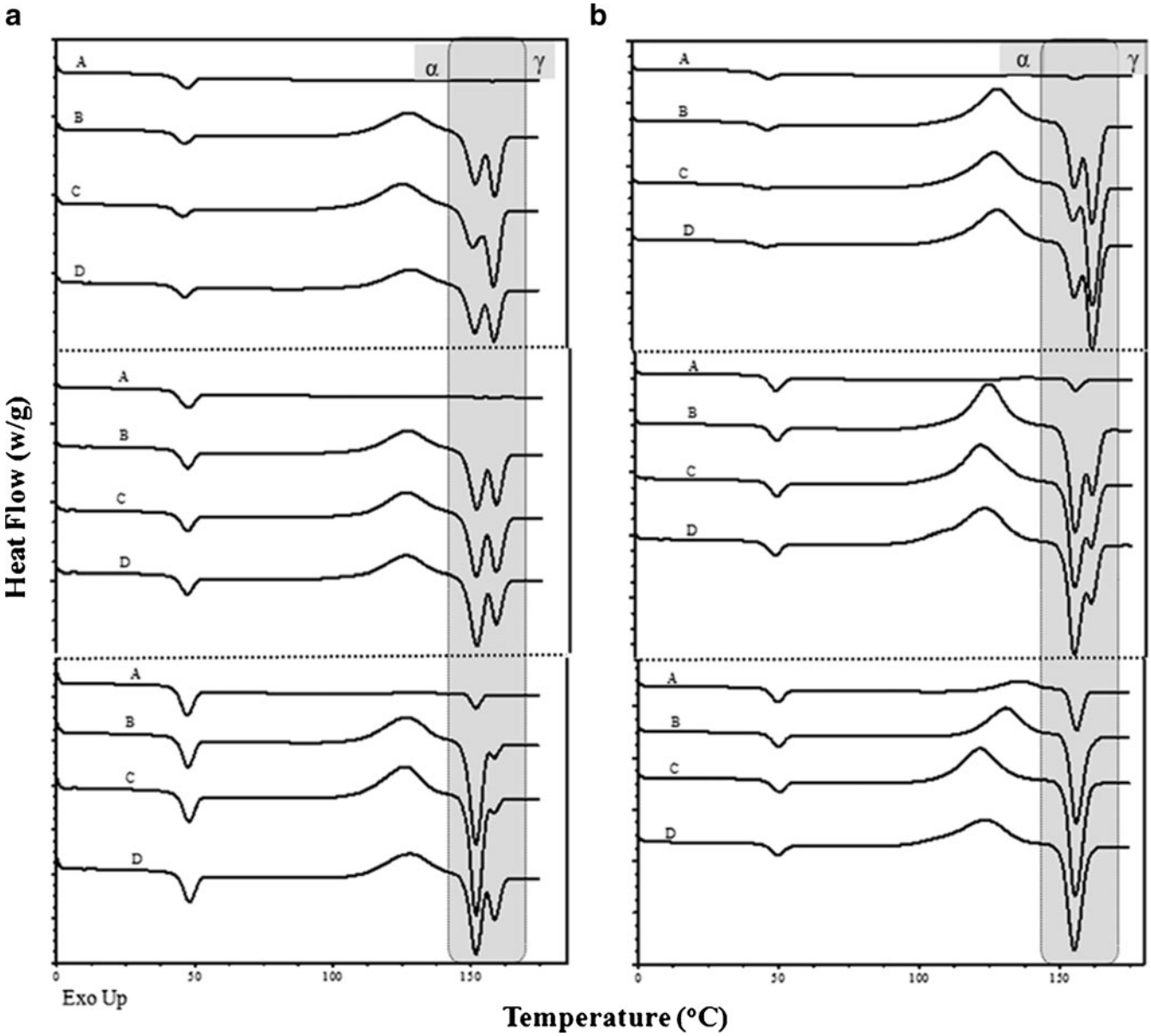

Fig. 4 Total heat flow signal for (**a**) slowly and (**b**) rapidly cooled samples (top to bottom: freshly prepared, aged for 5 and 10 days) for (A) uncompressed, compressed at 43.7 MPa for (B) 1 s, (C) 2.5 min and (D) 5 min dwell time.

(21). High pressure can increase the density of amorphous indomethacin even higher than supercooled liquid. The stabilization could be due to this pressure induced densification and/or pressure dependence characteristics of Johari-Goldstein secondary relaxation (β) (21). Okumura *et al.* also showed that the amorphous content is much higher at high pressure for ethanol slurry of amorphous indomethacin. In both cases the pressure was high and applied in closed chamber in every direction which can possibly lead to densification. This confinement can lead to a decrease in overall volume and molecular mobility which can ultimately stabilize the system (2). Tablet compression is a side constraint deformation in open system using a pressure which is not as high as in above mentioned studies. In this study the effect of compression was investigated using a setup which reflects pharmaceutical tabletting situation and showed that compression increases crystallisation of amorphous indomethacin even for the shortest dwell time (1 s). Unlike the previous studies, the effect of compression pressure on densification could be minimum or negligible due to the high density of glassy indomethacin. Mechanical stresses on glassy indomethacin induced by milling can also affect local molecular organization and high milling intensity could lead to crystallisation and then amorphization with milling time (8). Similarly compression can increase molecular mobility due to change in local molecular organization while exhibiting

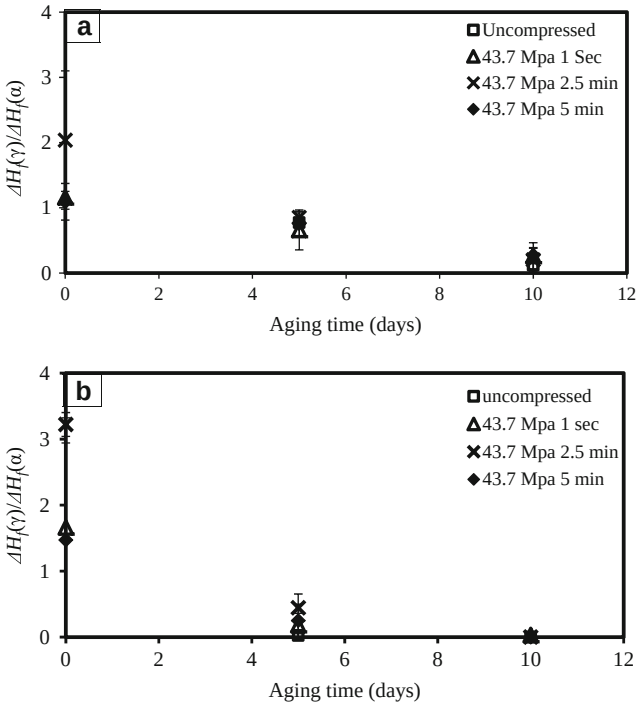

Fig. 5 Melting enthalpy ratio of γ to α-forms of (**a**) slowly cooled and (**b**) rapidly cooled amorphous indomethacin as a function of aging time (n=3).

deformation that increases the extent of crystallisation. Aged PMMA exhibited increased molecular mobility due to increase in the energy state after deformation (16). Capaldi *et al.* also showed enhancement of mobility of glassy polyethylene chains with deformation (12). However, a preliminary indentation study on glassy indomethacin showed that the system does not exhibit plastic deformation instead fragmentation (cracks) was observed already at minimal force (5 N). This observation supports that micro-cracks formed after compression may provide additional nucleation sites which lower activation energy and thereby facilitates crystallisation. The cumulative effect of enhanced molecular mobility and dominantly micro-cracks formation could facilitate overall crystallisation of amorphous indomethacin after compression. The effect of compression is also higher for rapidly cooled samples than slowly cooled samples as depicted in Fig. 6. As indicated on their recrystallization behaviour, rapidly cooled samples recrystallized to a higher extent compared to slowly cooled samples that can be an indication for the lesser stability of rapidly cooled amorphous indomethacin. But using compression as a stability predictor needs in-depth understanding on the origin of crystallisation of the glassy system and the mechanism/outcome of deformation. For example, stable amorphous solid dispersions of drug and polymer with conformational structure could be more affected by compression than those which are less stable systems devoid of any conformational structures (13). Glassy indomethacin prepared by different cooling rate has different densities. Wojnarowska *et al.* proposed that this difference in density could be the possible explanation for their stability difference (21). It was also reported that slowly cooled samples are stable against crystallisation even for 2 years and also exhibit better physical stability than quenched samples (21,22). However, Karmwar *et al.* reported that the physical stability of amorphous indomethacin increases with cooling

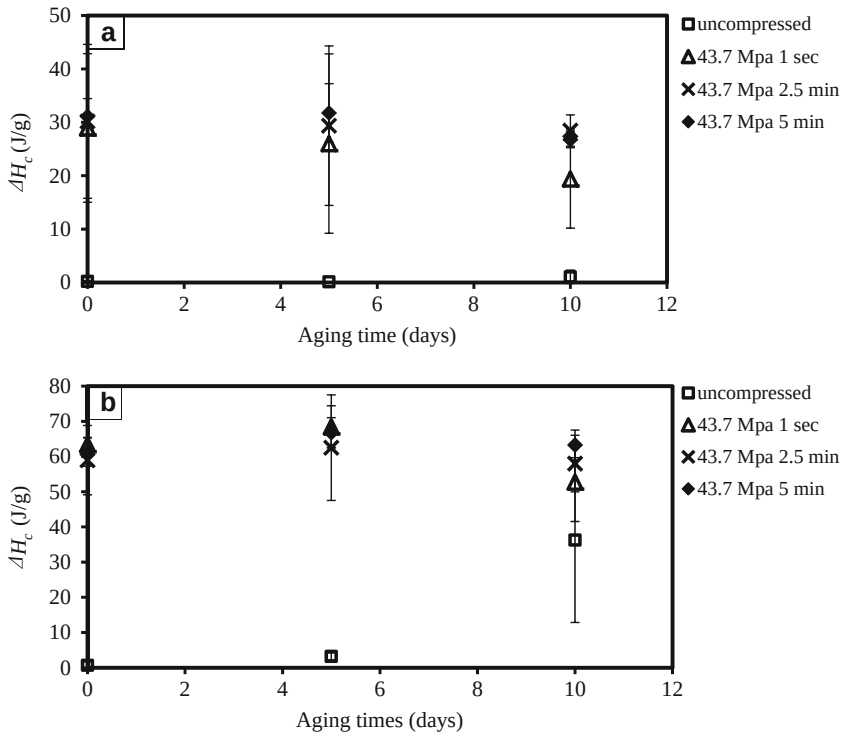

Fig. 6 Heat of crystallisation as a function of aging time for (**a**) slowly cooled and (**b**) rapidly cooled compressed (different dwell times) and uncompressed amorphous indomethacin (n=3).

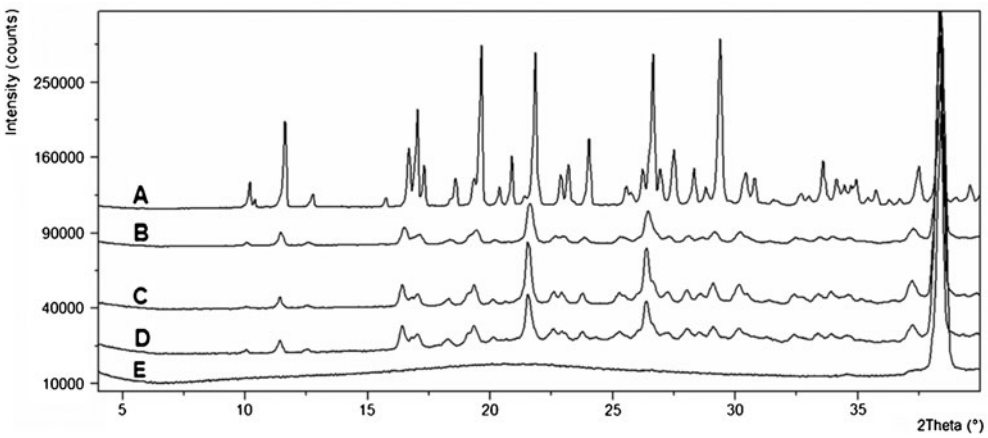

Fig. 7 PXRD pattern of crystalline indomethacin (A) and rapidly cooled amorphous indomethacin (aged for 10 days) further stored for 83 days (after compression and uncompressed): (B) compressed at 43.7 MPa for 1 s, (C) compressed at 43.7 MPa for 2.5 min, (D) compressed at 43.7 MPa for 5 min and (E) uncompressed.

rate (20). The finding from the present study clearly showed that compression has higher impact on physical stability for rapidly cooled samples compared to slowly cooled ones even though the response of glassy systems for compression might not be associated with their physical stability. Further screening studies are required to establish if compression studies can be regarded as a potential future stability predictor like humidity and temperature. Higher enthalpy relaxation of slowly cooled sample is apparently due to the higher duration of time during cooling to relax from non-equilibrium high energy state to equilibrium low energy state. Compression also showed significantly high enthalpy recovery on slowly cooled samples aged for five (1 s and 5 min DT) and ten days ($p<0.05$) but surprisingly it was not statistically significant for five days aged samples compressed for 2.5 min DT and fresh amorphous indomethacin. It is overt that the effect of compression pressure on molecular mobility could be negligible for freshly prepared glassy indomethacin as evidenced by similar enthalpy relaxation (Table I). Freshly prepared glassy indomethacin is already at high energy non-equilibrium state and compression pressure has less effect on relaxation behaviour of unaged melt-cooled samples (16). The insignificant difference in enthalpy recovery for five days aged samples before and after compression for 2.5 min DT does not have any physical meaning. Indeed, out of the three independent measurements at this dwell time there was one outlier (4.8 J/g) that was responsible for the high standard deviation of the mean value and the resulting non-significant difference. In general for aged melt-cooled glassy solids they will have PMMA like relaxation behaviour upon compression because of their higher density. However, porous systems like lyophilized and spray dried products usually show compression induced fast relaxation. Therefore, in freshly prepared melt-cooled amorphous indomethacin the effect of compression on relaxation behaviour could be insignificant as depicted in Table I especially for rapidly cooled amorphous indomethacin since it is already in high energy non-equilibrium state (16,17). The effect of compression will be more prominent for aged sample as indicated for slowly cooled sample aged for 5 (1 s and 5 min) and 10 days. However, it is important to determine either mechanical rejuvenation or densification is the prominent outcome of compression of glassy system to use enthalpy recovery as stability indicator.

Mechanical stresses instilled by sample handling and milling can also affect crystallisation of amorphous indomethacin. Samples handled under stressful conditions like grinding showed higher crystallisation that could be due to higher molecular mobility imparted by grinding which is also the case for compression. Bhugra *et al.* justified their observation based on the higher enthalpy recovery and TSDC results for ground sample (6). Understanding the effect of deformation on relaxation behaviour is also ambiguous and using enthalpy recovery as a measure of stability after deformation needs in depth understanding of the system. Aged PMMA was reported to have deformation induced rejuvenation to the higher energy state similar to freshly prepared melt-quench sample which means the enthalpy recovery value for aged PMMA after compression will be evidently lower (16). On the other hand lyophilized amorphous systems like sucrose showed fast relaxation behaviour after compression. The matrix structure of lyophilized products has higher porosity which attributes for their slow relaxation behaviour and upon compression the matrix structure was changed to non-porous solid which shortens the relaxation segment (17).

CONCLUSIONS

Compression of both slowly and rapidly cooled amorphous indomethacin induced and increased the extent of crystallisation drastically as evidenced by significantly high heat of crystallisation and lower crystallisation temperature. For freshly prepared samples compression led to significantly higher portion of α and γ-forms of indomethacin with the latter being more dominant. Compression of aged samples gradually led to a comparatively much higher proportion of α-form and for amorphous indomethacin prepared by rapidly cooling the melt and aged for 10 days only α-polymorph was

formed after compression. The transformation from γ-form to α-form dominant crystallisation was faster for rapidly cooled and compressed glassy indomethacin upon aging. Aging usually results in density equalization and densification of glassy amorphous indomethacin which ultimately leads to high degree of homogeneity. Crystallisation of this homogenous system will be more likely to a dominant or a single form as indicated for amorphous indomethacin stored for 10 days and then compressed. In addition it is worth establishing compression as a stability predictor stress condition for amorphous systems and establishing quality control measures for amorphous tablet formulation could be also very crucial.

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