Crystallisation Pathways of Polymorphic Triacylglycerols Induced by Mechanical Energy

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The aim of these studies is to establish sound scientific principles to guide nucleation rate and the selection of a desired polymorph via the application of mechanical energy - ultrasound (US) irradiation. When delivered to a metastable liquid, before the onset of nucleation and under constant temperature and supercooling conditions, the wave nature of this simple form of energy should be critical for defining different crystallisation pathways of polymorphic materials including polymorph selection. To test this hypothesis, we crystallized a melt-grown trilaurin (LLL), a typical polymorphic triacylglycerols (TGA’s), with and without US by using in-situ simultaneous synchrotron radiation time-resolved small-angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS), SAXS/WAXS. Without US application, both polymorphic forms β’ and β crystallized. With US treatment of the super cooled melt, the following effects were observed: (a) a marked decrease of induction times (b) an increased nucleation rate, and (c) selective crystallization of only β-form when crystallised at 25 and 30°C with input powers of 20 and 100 W and a sonication time of 2 s. Combining the existing knowledge on the dynamic nucleation of collapsing cavities and a qualitatively developed (P-T) phase diagram for the TGA’s, it was possible to describe, for the first time, the behaviour of the most important parameters and the events that characterize the crystallization of these systems. It was shown that the interplay of sonication and the temperature of supercooled melts are critical to the selection of a stable β-form.

1. Introduction

There has been an increasing interest in the application of ultrasonic waves to promote crystallisation from different phases [1, 2]. The application of power ultrasound during crystallisation (sono-crystallisation) may influence nucleation rate, morphology of particles, rate of crystallization as well as polymorphism [3-5]. The control of the above properties is of capital importance to the crystallisation of a wide range of materials used in processing industries dealing with foods, cosmetics, speciality chemicals, pharmaceuticals, etc. These characteristics can directly influence the efficiency of downstream processes such as separation, filterability, powder flow, caking, compaction, tabletting, and dissolution. In particular, the identification and control of polymorphism is essential to the process development of foods, speciality chemicals, and of special importance to the formulation and patenting of pharmaceutical materials.

In food industries, the following processes are closely related to polymorphic control: the processing of end-products made of fat crystals (such as chocolate, margarine and shortening), cream whipping and the separation of specific lipid materials from natural resources like oils, vegetable and animal fats, etc. In addition, it is well known that the polymorphism of these materials is quite susceptible to external factors governing crystallisation such as temperature, pressure and mechanical waves. This makes crystallisation of fats even more interesting to study since, in the course of crystallisation, the functional properties of fats can be manipulated using these parameters in a controllable manner, and hence industrial processes that lead to products of a desired quality. Thus, knowledge of the polymorphic state of polycrystalline fat is essential for the proper manufacture of fat containing products. In industry, the polymorphic control is usually
practiced by tempering or seeding [6]. The former is mostly applied to the chocolate or ice cream industries, whereas the latter refers to the use of certain additives such as seed crystals or templates. One of the challenges in the above processes is mastering polymorphic control by using ultrasonication.

A number of papers concerning sonocrystallization of fats have been published [7-9]. Quite recently, sonocrystallization has been examined in confectionery fats, indicating the possibility that tempering can be achieved by sonocrystallisation [4]. This work has been followed by the studies of the polymorphic behaviour of tripalmitin (PPP) and cocoa butter in the presence of ultrasound energy [5]. The main experimental findings can be summarized as follows: (a) the nucleation rate of PPP was enhanced and induction time was shortened by ultrasound application; (b) the polymorphic form V of cocoa butter was directly crystallized when ultrasound, under the optimal conditions of temperature and the duration of sonication, was applied. However, a key drawback of this study was that X-ray analysis of the crystallizing material has been done ex-situ using conventional X-ray facilities of limited power and resolution. Therefore, these results did not reflect the real-time crystallization process and as such, were not sufficiently informative. It is therefore quite obvious that such results inevitably lead to a speculative and somewhat uncertain interpretation of the crystallization process.

In this paper, the crystallisation behaviour of a typical triacylglycerol, trilaurin (LLL), is examined in-situ using synchrotron radiation time-resolved small angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) techniques. The results were considered within a developed framework based on a strong-coupling between a qualitative (T-P) diagram for LLL and the high pressure modified free energy for homogeneous nucleation. The potential for polymorph control has been investigated by using interplay between sonication power and supercooling of the melt.

2. Experimental Section

2.1 Materials

Trilaurin (LLL) was used as a sample and purchased from Sigma Aldrich (UK). It had a purity of more than 98% and it was used without further purification.

2.2 Methods

All experiments were conducted at the Station 6.2, at Daresbury SRS. This station enabled the performance of in-situ time-resolved small-angle X-ray scattering and wide-angle X-ray scattering (SAXS/WAXS) combined with a bespoke sonocrystallisation system, assembled from commercial and custom-made components (Fig 1).

The ambient of the analysing sample was manipulated and maintained using a custom-built crystallisation jacketed-cell with connections to two circulating thermostatic water-baths set at different temperatures. The origin of the water flowing in/out of the jacketed-cell was alternated between either water-baths depending of one’s need to either heat the sample (for melting) or cool down to the temperature of crystallisation. The windows of the cell are made from polyimide material (Kapton) of 25-μm thickness. The thickness of the sample that is exposed to the X-ray radiation was 1mm. The temperature of a sample was measured by a thermocouple, with an accuracy of ±0.5°C.
A sample of 30ml was put in the cell and melted by heating to 60 °C. Then, the sample was cooled down below its melting temperature to a desired crystallisation temperature by switching from hot to cool circulating water. A selected supercooling of the melt was achieved approximately within 5 to 10 min. From this moment, $t = 0$, simultaneous collection of WAXS and SAXS spectra began. These spectra were monitored and collected until the melt had entirely crystallised and evolved into the stable $\beta$-phase. Exactly the same procedure and supercoolings were used for sonication experiments. Sonication was initiated with an electronically controlled ultrasound generator, Model P100/3-20 (Sonic Systems Ltd, UK), once the melt achieved a desired crystallisation temperature. The duration of the sonication of the melt was of 2 seconds which was applied at various sonication powers. Spectra were taken at 10s interval. The induction times and polymorphic forms of the crystallised samples were determined by time-resolved simultaneous SAXS/WAXS measurements. Both these spectra are used to identify a particular polymorph, where SAXS defines the lamellar structure with long-spacing values and WAXS defines a corresponding short interplanar distance of a subcell [9]. SAXS, rather than WAXS, was used to measure induction times of the experiment due to its higher detecting system sensitivity. The camera length of SAXS was 3.5 m. For WAXS, this length was 0.3 m. Both SAXS and WAXS data analysis were calibrated according to the standard method using wet rat tail collagen and silicon. The system was operating at a wavelength of 0.14 nm.
3. Results

3.1 Polymorphic forms of LLL

There are three known polymorphic forms of LLL crystals, $\alpha$, $\beta'$ and $\beta$ with $\alpha$-form being the least stable and $\beta$-form the most stable. The main difference between these polymorphs is found in the angle of tilt from the base plane in the crystal lattice (Fig. 2a). Figure 2(b) shows the crystal physical appearance of $\beta'$ and $\beta$-forms of LLL under an optical microscope. The melting points and the angles of tilt for the three polymorphs of LLL are presented in Table 1.

![Polymorphic lamellar structure of LLL.](image)

**Figure 2.** (a) Polymorphic lamellar structure of LLL. (b) Optical microscope images of LLL $\beta'$ and $\beta$-forms.

![Optical microscope images of LLL $\beta'$ and $\beta$-forms.](image)

**Table 1.** Melting points and the calculated angle of tilt of the lamellae structure in LLL [10].

| Polymorphic Forms | $\alpha$-form | $\beta'$-form | $\beta$-form |
|-------------------|---------------|---------------|--------------|
| Melting point, $T_m$ ($^\circ$C) | 15.0 | 35.0 | 46.5 |
| Angle of tilt, $\theta$ ($^\circ$) | 90$^\circ$ | 66$^\circ$ | 63$^\circ$ |

3.2 Studies of Induction Times and Polymorphic Transformation

Figure 3(a) shows typical time-resolved synchrotron radiation X-ray diffraction SAXS and WAXS spectra from the crystallising LLL melt at constant crystallisation temperature, $T_{\text{cryst}} = 25^\circ$C, without sonication. The spectra were taken at 10s interval. However, for clarity of presentation and to avoid a highly dense layout, not all spectra are reproduced in the Figures. The time is regarded as zero, $t = 0$ s, at the moment when a desired crystallisation temperature, in this case $T_{\text{cryst}} = 25^\circ$C, is achieved. The driving force (supercooling) for melt crystallisation is the difference between the melting point of a substance and the crystallisation temperature. In this case, the degree of metastability for each polymorphic form is:

$$\Delta T_{\text{cryst}}^{\beta'} = T_{m}^{\beta'} - T_{\text{cryst}}$$

(1)

and

$$\Delta T_{\text{cryst}}^{\beta} = T_{m}^{\beta} - T_{\text{cryst}}$$

(2)

where $T_{m}^{\beta'}$ and $T_{m}^{\beta}$ are the melting temperatures of $\beta'$ and $\beta$ respectively.
Figure 3. Time-resolved SAXS/WAXS data of crystallising melt of LLL at $T_{cryst} = 25$: (a) without ultrasound; (b) US input power = 5W, 2s sonication time and; (c) US input power = 100W, 2s sonication time.
The diffracted peaks observed in SAXS Figure 3(a) confirmed that both β’- and β-form crystallised, with β’-form crystallised first. Induction time was determined as the length of time measured from the moment the temperature of the system has cooled down to the crystallisation temperature, \(T_{\text{cryst}} = 25^\circ\text{C}\) to the moment at which the first appreciable peak of the SAXS spectrum was detected. The induction time determined in this way was for β’-form 280 s and for β-form 540 s. This agrees with the Ostwald rule of stages which implies that the preferred polymorph to crystallise first is the less stable one.

Figure 3(b) shows the time-resolved WAXS and SAXS spectra for crystallisation of LLL obtained at an input power of 5 W while the other external factors were kept the same as those in the non-sonicated case. Upon comparison between the SAXS spectrum of the sonicated and that of non-sonicated crystallising melts, it becomes clear that both polymorphic forms crystallised, with the metastable β’-form arising first. However, unlike in non-sonicated case, the induction time in the US irradiated melt is 250 s for β’ and 420 s for β. Both values are appreciably smaller than those for nonsonicated melt. This suggests that even a small input power of the ultrasound may have a quite strong effect on increasing the nucleation rate, \(J\), for both phases (\(J \sim 1/\tau\)). The metastable form did not last for a long and disappeared quickly when β-form begins to crystallise.

When a considerably higher input power of 100 W was applied under the same other external conditions, the corresponding SAXS/WAXS spectra showed two markedly new effects caused by the applied sonication power (Fig 3(c) and 3(d)). The first one is manifested through the prevention of the nucleation of the metastable β’ (selection of β), while the second one through an apparent shift in the reflections (110) and (100) of the β WAXS spectra towards slightly larger \(2\theta\). It is important to notice that these shifts were only observed in the experiments where ultrasound energy was applied and only the stable β-form crystallised. A similar effect, with a selection of only stable β form, was observed at the input
power of 20 W (WAXS/SAXS spectra not shown here), but with a relatively longer induction time of 140 s compared to that of 90 s obtained for the input power of 100 W.

Finally, we find that crystallisation temperature may have a significant effect on the induction times of the two polymorphic phases. Using the same external conditions as those for $T_{\text{cryst}} = 25^\circ\text{C}$, but now with a higher crystallisation temperature, $T_{\text{cryst}} = 30^\circ\text{C}$, we find that the general features of the effect remain unchanged, but the inductions times become somewhat bigger compared to those obtained at $T_{\text{cryst}} = 25^\circ\text{C}$ (Table 2).

Table 2 summarizes induction times and the corresponding external conditions of supercooling, crystallisation temperature, input power of the ultrasound and its duration, under which the experiments were conducted.

| Crystallisation temperature, $T_{\text{cryst}}$ | Induction time | $\beta'$-form | $\beta$-form |
|-----------------------------------------------|----------------|---------------|--------------|
| $T_{\text{cryst}} = 25^\circ\text{C}$        | Supercooling, $\Delta T_{\text{cryst}}$ | 10°C          | 21.5°C       |
| $\tau$                                        | US = 0s        | 280s          | 540s         |
|                                               | US = 2s, 5W    | 250s          | 420s         |
|                                               | US = 2s, 20W   | 140s          |              |
|                                               | US = 2s, 100W  | 90s           |              |
| $T_{\text{cryst}} = 30^\circ\text{C}$        | Supercooling, $\Delta T_{\text{cryst}}$ | 5°C           | 16.5°C       |
| $\tau$                                        | US = 0s        | 340s          | 580s         |
|                                               | US = 2s, 5W    | 300s          | 460s         |
|                                               | US = 2s, 100W  | 240s          |              |

The overall observations presented in this section lead to the following findings: (1) the induction time of either metastable or stable polymorphic form of LLL seems to be quite susceptible to the input power of applied sonication; our experiments showed that even the applied power of 5 W for 2 s is sufficient to reduce induction times substantially compared to those without sonication, (2) the selection of the stable polymorphic form $\beta$ is achievable at higher levels of the input power at the same other external conditions of crystallisation, (3) the induction time of the selected stable form reduces with input power and temperature and (4) WAXS spectra of ultrasonically selected $\beta$ shows at the initial stage of the growth of this form the peak shifts of the (110) and (100) reflections.

4. Discussion

In the following we shall try to give an account for the key findings, (1) to (4), presented in the previous section.
4.1 Consideration of Induction Time

4.1.1 Qualitative Temperature-Pressure Diagram

The crystallisation of LLL under the influence of ultrasound, in general, is shown to reduce the induction times of the crystallisation of the polymorphs. To analyse this finding we make use of a qualitative temperature-pressure (T-P) diagram. Since this diagram is still not available for any TGAs including LLL, we shall define it qualitatively by using the Clapeyron equation and relevant experimental data concerning this material. This equation applies to equilibrium between any two phases and is given by

\[
\frac{dT}{dP}_{\text{equiv}} = T \frac{\Delta V}{\Delta H}
\]

where \(\frac{dT}{dP}_{\text{equiv}}\) is the change in the melting temperature \(T\) with pressure \(P\), \(\Delta V\) is the corresponding molar volume change due to melting and \(\Delta H\) is the enthalpy of melting. For most substances including fats, \(\frac{dT}{dP}_{\text{equiv}}\) is positive. It is assumed that this derivative is constant for a wide range of the (T-P) phase diagram. The thermodynamic properties characterising the right side of eq. 3 are experimentally known apart from \(\Delta V\). The latter one can be calculated as \(\Delta V = M \left( \rho_s - \rho_l \right) \left( \rho_s \cdot \rho_l \right)^{-1}\), where \(M\) molar mass, \(\rho_s\) is the density of solid phase and \(\rho_l\) is the density of an isotropic liquid phase (melt).

The density of any phase of a fat is determined from an experimental dependence that describes the change in the density of a particular phase as a function of the number of carbon atoms present in the acyl-chains in the fat molecule (Figure 4). These dependencies were obtained by applying a least-squares fitting procedure to the available experimental data [10-13].

![Figure 4. The density of fats as a function of the number of carbon atoms present in their corresponding acyl-chains. Solid and broken lines are the least-square fitted curves for the three physical states shown.](image-url)
To get a qualitative idea about (T-P) phase diagram for both $\beta'$ and $\beta$, we express Eq. (3) in its explicit form for each polymorph and find the ratio

$$\frac{dP}{dT}_{\text{equil}} = \frac{T_m^{\beta'} (\Delta V)^{\beta'}}{(\Delta H)^{\beta'}} = \frac{T_m^{\beta'} (\rho_s^{\beta'} - \rho_l^{\beta'})}{(\Delta H)^{\beta'} \cdot \rho_s^{\beta'}}$$

(5)

When the corresponding experimental values $T_m^{\beta'}$, $\rho_s^{\beta'}$, $\rho_l^{\beta'}$, $(\Delta H)^{\beta'}$, $T_m^{\beta}$, $\rho_s^{\beta}$ and $(\Delta H)^{\beta}$ are substituted into this equation, the calculated ratio of the gradients is 1.30. The fact that this value is greater than 1 suggests that an equilibrium straight line of the $\beta'$ form should be steeper than that corresponding to $\beta$. This suggestion leads us to a possible qualitative (T-P) diagram (Figure 5). Although this diagram was worked out by using thermodynamic parameters for LLL, it can be easily demonstrated that its general appearance would remain the same for any other fat from the family of TGA presented in Fig 4. Therefore, Fig 5 should reflect the equilibrium between the solid and liquid phases for both polymorphs of a fat over a significantly large range of temperatures and pressures. Due to the difference in gradients between the two polymorphs, the straight equilibrium lines are expected to cross one another at some point C, defined by a particular pair of temperature and pressure.

When ultrasound is applied to a liquid (melt), two events occur simultaneously; (i) the mechanical waves of ultrasound induce periodic change in the liquid [14] and (ii) the liquid absorbs energy from the applied ultrasound [15].

Table 3 summarises the values of the densities found from Fig 4 for each form of LLL, the calculated corresponding values of $\Delta V$ and experimentally known changes of the enthalpy of melting for the two polymorphs.

**Table 3. Thermodynamic properties of polymorphic forms of LLL.**

| Polymorph LLL | $\beta'$ | $\beta$ |
|---------------|----------|---------|
| $\Delta H$ (kcal/mol) | 19.4 | 19.5 |
| Molar volume change, $\Delta V \times 10^{-6}$ (m$^3$/mol) | 106.3 | 123.1 |
| Density of solid phase, $\rho_s$ (g/cm$^3$) | 1.03 | 1.06 |
| Density of liquid (melt) $\rho_l$ (g/cm$^3$) | 0.88 |
If the applied ultrasound wave has a pressure amplitude that is higher than that of the ambient pressure of the liquid, the transient cavitation bubbles will be formed then collapsed followed by the development of extremely high pressure in their vicinity [7]. An increase in the local pressure consequently causes a rise in temperature. The effect of local changes in pressure and temperature in the crystallisation of LLL is discussed in details in the following two sections.

4.1.2 Change in Local Pressure

The mechanism for the collapse of a cavitation bubble is still not fully understood. It is less clear how a very high pressure localised in space and time develops in the vicinity of the collapsing bubble. Initially, it has been speculated that a bubble overexpands in the negative part of an applied acoustic wave, caves in sharply in the positive half to conceivably 1% of its initial radius, and then rebounds, sending out a pressure pulse (shock wave) [16-18]. These speculations have been recently confirmed experimentally to a considerable extent [19]. However, the measurements of the real pressure and temperature in a collapsing bubble and in the consequently developed shock-wave still remain unresolved. Therefore, to get some idea about the order of magnitude of the parameters that characterise these transient phenomena, we have to rely on the data that result from different models [20-22]. Estimated calculations for a moderately supercolled liquid show that at a point approximately $10^{-4}$ cm from the bubble wall (of diameter $\sim 10^{-5}$ cm the liquid is subjected to average pressure of the order of 10 kbar over a period of $10^{-9}$ s [17]. It has been shown that at positive pressure pulses of this order or higher, nucleation in supercooled liquids is likely to be initiated [20-23]. This kind of nucleation has often been considered as a dynamic nucleation, because it is caused by the relative motion of the crystallising liquid [24]. In the following section, we shall try to give an account for this phenomenon in sonicated LLL melt using a combination of the classical theory of homogeneous nucleation of a supercooled liquid [25] and the melting point shift [26] whose magnitude is related to the pressure change via the Clapeyron equation.

![Figure 5. Qualitative Temperature vs. Pressure diagram for $\beta'$ and $\beta$-forms of fats.](image-url)
According to the classical theory for homogeneous nucleation of an undercooled liquid [23, 25], the rate of nucleation, $J$, is given by:

$$J = \left( \frac{nkT}{h} \right) \exp \left( -\frac{\Delta G_a}{kT} \right) \exp (-\Delta W)$$  \hspace{1cm} (6)$$

where $n$ is the number of molecules/cm$^3$, $k$ is the Boltzmann constant, $T$ is the absolute temperature, $h$ is the Planck’s constant, $\Delta G_a$ is the activation energy for a molecule to cross the liquid crystalline face, $\Delta W$ is the height of the free-energy nucleation barrier for a cluster to overcome it and grow irreversibly as a crystal. This barrier is given by:

$$\Delta W = \frac{16\pi}{3} \frac{\gamma^3}{kT\rho^2\Delta H^2 \left( \frac{\Delta T}{T_m} \right)^2}$$  \hspace{1cm} (7)$$

in which $\gamma$ is the free energy between the liquid and crystalline interface, $\rho$ is the density of the solid phase, $\Delta H$ is the molar enthalpy of melting, $T_m$ is the melting temperature $\Delta T = T_m - T = T_m - T_{cryst} = \Delta T_{cryst}$ is the supercooling. In addition to this, we assume that the structure of a nucleation cluster of either phase is the same as that of the corresponding crystal phase.

As we saw earlier, application of ultrasound to a metastable liquid results in high pressure pulses generated in the vicinity of collapsing bubbles in the undercooled melt. As a consequence, a sudden onset of nucleation is likely to occur in these small high pressure volumes of the liquid because of the displacement of the melting point [20]. More specifically, an increase of pressure towards its own maximum in such a small volume of the melt, may well result in the local increase of enthalpy, solid-liquid interface energy, density and melting point. If $\Delta P$ is the difference between the pressure in the vicinity of a collapsing bubble and the ambient (atmospheric) pressure, then the new enthalpy at the new melting point is given by $\Delta H^{US} = \Delta H(1 + \delta)$, where $\delta = \frac{\Delta P\Delta V}{\Delta H}$ in which $\Delta V$ is the difference in specific volumes between the undercooled melt and the crystalline phase. A further consequence of the ultrasound perturbation is reflected on the changes of other thermodynamic quantities; the internal density of a nucleus and the corresponding interfacial free energy between the nucleus and the liquid turn into new values, $\rho^{US}$ and $\gamma^{US}$, respectively. These changes are given by $\frac{\rho^{US}}{\rho^0} = \frac{(1 + \delta)\rho}{\rho^0}$. Similarly, a displaced melting point due to sonication ($T_m^{US}$), due to sonication is given by $T_m^{US} = T_m(1 + \delta)$. Hence, it is easy to show that $\frac{\Delta H^{US}}{T_m^{US}} = \frac{\Delta H}{T_m}$. Therefore, the new height of the free-energy nucleation barrier in the vicinity of the collapsing cavity becomes

$$\Delta W^{US} = \frac{16\pi}{3} \frac{\gamma^3(1 + \delta)^3}{kT^{US}\rho^2\Delta H^2 \left( \frac{\Delta T^{US}}{T_m} \right)^2}$$  \hspace{1cm} (8)$$
where $T^{US}$ is the local change in the temperature of the melt and $\Delta T^{US} = T^{US} - T^{US}$ is the corresponding undercooling. Both $T^{US}$ and $\Delta T^{US}$ can be obtained from an isothermal approximation in a qualitative $(T-P)$ diagram. For the isothermal process: $T^{US} = T = T_{cryst}$. Using the Clapeyron equation and the straight melting lines (Figure 5), we get $\Delta T^{US} = \Delta T_{cryst} + \delta T_{m}$, where $\Delta T_{cryst}$ is the initial supercooling and $\delta T_{m}$ results from $\Delta P$.

Equation (8) can be now expressed as

$$\Delta W^{us} = \frac{16\pi}{3} \frac{\gamma^3(1+\delta)^3}{kT_{cryst}\rho^2\Delta H^2 \left(\frac{\Delta T_{cryst}}{T_m} + \delta\right)^2}$$

(9)

This expression gives an estimate of the free-energy barrier for nucleation in a very small volume of very high-pressure around the collapsing bubble created by applied sonication. For further analysis it is appropriate to consider a ratio of this equation and equation (7) which gives a free-energy barrier for nucleation from non-sonicated melt:

$$\frac{\Delta W^{us}}{\Delta W} = \left(\frac{\Delta T_{cryst}}{T_m}\right)^2 \frac{(1+\delta)^3}{\left(\frac{\Delta T_{cryst}}{T_m} + \delta\right)^2} = \left(\frac{\Delta T_{cryst}}{T_m}\right)^2 \frac{1}{\left(1 + \frac{\Delta P\Delta H}{\Delta H}\right)^3}$$

(10)

Replacing the corresponding experimental values from tables 1, 2, 3 and taking $\Delta P \approx 10\text{kbar}$, this ratio can be then estimated for either of the two polymorphs of LLL. A simple calculation shows that for both $\beta'$ and $\beta$, $\frac{\Delta W^{us}}{\Delta W} \ll 1$. This implies that the height of the free-energy barrier for a cluster to overcome, before it stands a chance of surviving and growing irreversibly as a crystal, is much smaller in the melt around the collapsing bubble than that in the non-sonicated melt. Referring back to Equation (6), reduction in the free-energy barrier for nucleation around the collapsing cavity can trigger nucleation in one or more spots, resulting in an increase of the nucleation rate, $J$. Since $J \propto \frac{1}{\tau}$, it implies reduction in induction times [27]. This agrees with the results obtained and presented in Table 2 where the induction times are reduced remarkably upon application of ultrasound for crystallisation of LLL. However, this model does not explain why in some cases, only the stable beta form was crystallised. The proposed model indicates that cavitation is a necessary but not sufficient condition for dynamic nucleation; additional factors need to be taken into account.

At this stage we argue that factors such as the duration of the final stages of collapsing cavities, the lifetimes of the subcritical nuclei and stability of subcritical nuclei may play a critical role in the nucleation of a particular polymorph. In a fairly supercooled melt similar to those used in our experiments, the duration of the final stage of a collapsing bubble, at which a high pressure pulse is developed in its vicinity, is of the order of nanoseconds [9, 22]. This suggests that in order to generate a particular polymorph, this time should be of the order or longer than an average lifetime for a cluster (subcritical nuclei). The lifetime scales of the less stable $\beta'$ clusters are probably too short compared to the duration of the collapsing cavities.
and therefore, β' clusters were dissolving before achieving their critical size. Comparing to the more stable β clusters which are also subjected to the same local change of pressure, it is reasonable to assume that an average lifetime of this type of clusters is comparable or bigger than the duration of the final stage of the collapsing bubble. Apparently, in this case, application of sonication acts as a catalyst which promotes the nucleation and hence irreversible precipitation of the β form.

At the end, we give a brief comment on how locally induced ΔP might influence ΔGa, the activation energy for a molecule to cross the liquid interface. It is likely that a locally increased pressure will result in an increase of the viscosity of the supercooled melt. For relatively small molecules such as LLL, this effect should be negligible while for relatively large molecules, an increase in pressure could significantly reduce the nucleation rate \[28\]. However, since the induction times observed in this work (Table 2) are pronouncedly reduced due to sonication, we believe that ΔGa has not been influenced noticeably by ΔP.

### 4.2 Effect of Temperature

A comparison of induction times at the crystallisation temperature of 25°C with their counterparts obtained at \(T_{\text{cryst}} = 30 \, ^\circ\text{C}\) (Table 2), shows a quite clear increase of induction times with temperature regardless of the melt was sonicated or not. These findings are in a good agreement with equations (6) to (9). In order words, under the same conditions of sonication, the closer the crystallisation temperature to a melting point of either polymorph, the lower the corresponding supercoolings and hence, the higher the free-energy barriers for the nucleation of both β' and β form. The longer induction times observed at higher temperature imply that the thermal energy may have a significant destructive effect on the nucleation processes of both polymorphic forms.

It is important to mention that, in principle, the stability of an externally imposed crystallisation temperature can be affected by inevitably generated heat that stems from the application of sonication and the crystallisation itself.

The heat generated by sonication is caused by the dissipation of acoustic energy and the heat formation in the highly compressed gas in the cavitation bubbles. The former one is a consequence of the interaction between the mechanical wave and the liquid, which is manifested through viscous losses, heat conduction losses, and the losses related to internal molecular processes \[29\]. The latter one is transferred into the liquid shell between the collapsing bubbles and the liquid by radiation and conduction.

Finally, the last contribution to the overall increase of temperature may come from the precipitation of the solid phases which is accompanied by the release of the heat of the heat of crystallisation.

The observed temperature increase due to the above factors had an appreciable value of approximately 2°C for a relatively short period of time during and after the sonication and then would cease to exist for the rest period of crystallisation. The contribution of this effect is rather difficult to separate. We believe that the overall heat corresponding to the observed temperature increase, apart from decelerating growth of the already nucleated melt, should not have any significant effect on the observed differences in the induction times of the nucleation processes because all experiments were performed at same external conditions of the input sonication power and its duration and above all, the temperature increase was almost the same.
4.3 Shifts in β-form WAXS Peaks

The peak shifts in WAXS spectra of different TGA’s have been observed earlier at different – nonsonicated conditions [30-32]. These authors associate the peak shifts occurrence in the course of crystallisation to a possible existence of either new metastable phases or remains of β whose growth precedes the growth of the stable β phase. However, a great deal of controversy still remains regarding the existence of possible crystalline phases and the mechanisms for phase transformations. Apparently, the main reason for this lies in a quite limited knowledge of a three-dimensional atomic-level crystal structure of the family of these materials [11]. This has been partly because of problems in growing TGA single crystals to a sufficient size and perfection for X-ray diffraction studies. A second complication relates to the fact that metastable polymorphs including β have a strong tendency to transform to the more stable β form. As a consequence of these two factors, a quantitative characterisation concerning crystal lattice distortion of these possible transient polymorphs is not available at present. Therefore, there is a significant amount of uncertainty of whether peak shifts are caused by new polymorphs or by a pronounced imperfection of a stable β-form at its initial stage of growth.

At this stage we argue that the peak shifts of the (110) and (100) reflections of the WAXS spectra of the β-form of LLL (Fig 3 (c) and (d)) may be influenced by a marked presence of the strain developed in the particles of this phase just after they have been nucleated. This phenomenon is well known and is typical for very small particles in inorganic and organic systems [33-34]. This strain is usually caused by liquid inclusions captured by morphologically unstable facets of the growing particles [35]. A shifted reflection may be a result of the X-ray diffraction from an ensemble of strongly structurally defected particles and the presence of the traces of amorphous liquid inclusions in them. At the later stage of growth, it is quite likely that liquid inclusions will solidify and that the previously induced strain will relax on the growing facets by forming dislocations in the particles. These processes are time dependent and accompanied by a pronounced increase in crystal perfection, which finally leads to the peak positions of undistorted β particles. An additional reason that leads us to the proposed scenario lies in the fact that the position of the SAXS spectra (Fig 3 (c)) remains unchanged throughout the crystallisation process. Due to the lack of atomic crystal structure and consequently the anisotropy of elastic constants within the material, the question of why these particular reflections are most affected remains open.

5. Conclusions

The effects and mechanisms by which ultrasound energy may influence the crystallisation of polymorphic LLL have been examined by synchrotron radiation time resolved SAXS/WAXS measurements. A striking reduction of the induction times (and an increase in the corresponding nucleation rates) has been observed in the sonicated supercooled melts. These results are explained in the light of two major events that may be critical for the observed effects: (1) the ultrasound-stimulated nucleation occurred in the high pressure vicinity of the collapsing bubbles because of the melting point shift and the consequent increase of supercooling and (2) a pronounced decrease of the free energy barrier for the nucleation compared to that without sonication. At the fixed conditions of sonication power and its duration, it was shown that at the higher crystallisation temperature the longer
induction times were needed for the onset of nucleation events. At the initial stage of precipitation, the selected crystallisation temperatures were slightly and to the same extent increasing, by 2°C on average, due to the heat generated by the absorption of the applied ultrasound energy and the crystallisation heat. In spite of fact that a total heat generated in this way is inevitable and almost the same regardless of the crystallisation temperature, the corresponding increase of this temperature should not have any fundamental influence on the nucleation behaviour of the two polymorphs versus the externally imposed temperature. It has also been shown that is possible to select the stable polymorph phase β of LLL. The selection of this phase requires the interplay between sonication power, crystallisation temperature and supercooling. At the end, an account for the peak shifts of the (110) and (100) reflections of the β-WAXS spectra has suggested and related to the presence of anysotropically distributed unrelaxed strain caused by the capture of the liquid inclusions at the initial stage of growth.

We believe that apart from making some progress in clarifying the presented complexity, the above findings suggest new research challenges to be followed. An ultimate goal is to develop a theoretical model, and more importantly, one with a certain degree of predictability of a desired polymorph. At present, none of these two is available. To achieve this we need a better understanding of the following issues: (i) (T-P) phase diagram for β' and β forms, (ii) stability (lifetime) of the nuclei of these polymorphic forms as a function of temperature, (iii) the mechanism and lifetime of collapsing cavities, a basic mechanism of the dynamic nucleation in the vicinity of a collapsing bubble, (iv) crystal structure on an atomic level of most of TGA and (v) structural defects and thermal behaviour of these materials. The work presented here should be a generic one and as such applicable not only to LLL, but also to any TGA.

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