# Sonocrystallisation of Lactose in an Aqueous System

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## Abstract

Although research on sonocrystallisation of lactose has been reported in the literature (yield and crystal size), the effect of ultrasound variables on nucleation and growth rate of lactose have not been studied. In this study, lactose crystallisation with ultrasound was investigated and compared to mechanical agitation using the induction time method at 22 °C. Ultrasound had a significant effect in reducing induction times and narrowing the metastable zone width but had no effect on individual crystal growth rate or morphology. A rapid decrease in induction time was observed up to 0.46 W $g^{-1}$ power density. Sonication up to 3 min decreased the induction time but no further reduction was observed beyond 3 min. It was not possible to generate the nucleation rates achieved by sonication using agitation alone. One minute sonication at 0.46 W $g^{-1}$ power density followed by continuous stirring was found to be the optimum under the experimental conditions tested.

## 1. Introduction

High intensity sound waves passing through solutions generate acoustic cavitation which results in micro-bubbles present in solution to grow in size and implode violently, generating localised high temperatures and pressures (Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010). Cavitation is capable of altering physical, mechanical or chemical properties of materials. The ability of the ultrasound to cause cavitation depends on many factors, such as the frequency and intensity of ultrasound, properties of the liquid and ambient conditions (T. Mason & Lorimer, 2002). Ultrasonic irradiation and cavitation in liquid and solid-liquid systems can enhance reaction rate and product yield and facilitate mass transfer and reactant diffusion (Li, Li, Guo, & Liu, 2006). Studies on other systems have shown that sonocrystallisation generally exhibits four features which do not occur in crystallisation without sonication. These are faster primary nucleation, ease of nucleation, initiation of secondary nucleation and production of smaller and purer crystals (Luque de Castro & Priego-Capote, 2007). For example, Li *et al*. (2006) reported that by varying ultrasound power, duration and solution volume, the mean size, crystal size distribution (CSD) and crystal shape can be perfectly controlled in spectinomycin hydrochloride crystallisation.

Lactose is the major carbohydrate in milk and the major constituent of many concentrated and dried milk and whey products. Lactose is crystallised from whey or permeate concentrated up to 50-70 % by evaporation. Crystallisation is initiated either by flash cooling or by seeding with a small quantity of lactose crystals, usually in batch crystallisers cooled down to a predetermined temperature. Depending on the specific product or processing objective, the desired lactose crystal size varies. For example, large crystals are wanted in lactose production to enable recovery while crystals less than 20 μm are required for spray dried whey powder. Improved control of the lactose crystallization process has particular significance for the dairy industry (Westergaard, 2010). In lactose manufacture, ultrasonication is potentially an alternative way of “seeding” to induce crystallisation and produce smaller crystals with a narrower CSD and shortened time of crystallisation. Application of ultrasound was reported to increase the yield of lactose crystallisation with ethanol as an anti solvent (Bund & Pandit, 2007a,b,c),

(Kougoulos, Marziano, & Miller, 2010), in acetone as an anti solvent (Patel & Murthy, 2009) in aqueous solution and in viscous glycerine solution (Dhumal, Biradar, Paradkar, & York, 2008). However, the effect on nucleation and growth rate and the impact of ultrasound variables has not been reported in a simple aqueous system.

Nucleation is the formation of a new solid phase from a supersaturated solution and it significantly affects the crystallisation process and properties of the final product. Nucleation rate can simply be explained as the change in the number of particles in solution with respect to time. The number of particles can be measured by different methods such as light scattering, direct particle counting and turbidity measurements (Gherras & Fevotte, 2012). The presence of solid particles in solution changes transmission of light; therefore absorbance measurements with UV-VIS spectroscopy can be used to estimate the number of particles in solution when correlated with direct counting of particle numbers. Turbidity measurements were reported to be an inexpensive, quick and reliable method for the measurement of induction time (Kuldipkumar, Kwon, & Zhang, 2007). The induction time has been used by many researchers for a variety of unseeded aqueous solutions to determine nucleation rate. It is defined as the time elapsed from the creation of supersaturated solution and the detection of a new phase and it was interpreted as either the appearance of first crystals or as a point at which the number density of crystals reached a predetermined value (Kobari, Kubota, & Hirasawa, 2012). Mcleod (2007) reported nucleation rates of lactose in aqueous solutions and simulated whey permeate by measuring absorbance at 550 nm by UV-VIS spectroscopy. The time taken to reach an absorbance of 0.1 was taken as the critical time and the nucleation rate was calculated dividing the number of particles with time taken. Up to absorbance of 0.2, the nucleation rate calculated did not change (Mcleod, 2007).

In this research, the impact of ultrasound on lactose nucleation and growth rate was investigated in aqueous systems using absorbance measurements and the induction time method. The effects of concentration, power density and sonication time were examined with sonication and compared with mechanical agitation.

## 2. Materials and Methods

Food-grade α-lactose monohydrate (Murray Goulburn Co., Melbourne, Australia) with 99.6 % purity was used in all experiments. Lactose solution was prepared by heating lactose in deionised water to 70 °C with constant stirring on a hot plate until all solids were dissolved. The solution was cooled down to 22 °C over four hours on the bench and filtered through a 0.8 μm membrane filter (Millipore, Type AAWP, Billerica, MA, USA). The weight of lactose solutions used was 300 g and all experiments were performed at 22°C. Relative $\alpha$–lactose supersaturation (S) and absolute alpha lactose supersaturation ($C_\alpha$-$C_{\alpha s}$) were calculated using the equation given by Visser (1982) and Butler (1998) where C is lactose concentration, $C_s$ is the equilibrium solubility of lactose, F is the factor accounting for β-lactose depression of α-lactose solubility and $K_m$ is the equilibrium constant describing the equilibrium ratio of β to α-lactose (Visser, 1982). The $C_s$, F and $K_m$ values at 22 °C are 19.90 g 100 $g^{-1}$, 1.587 and 0.31 respectively.

$$S = \frac{C_\alpha}{C_{\alpha s}} = \frac{C}{C_s - FK_m(C-C_s)} \quad \text{and} \quad C_\alpha - C_{\alpha s} = \frac{C - C_s + FK_m(C-C_s)}{(1+K_m)}$$

The number of particles in the solution was determined by correlating the absorbance of solutions (the UV/VIS spectrophotometer, Model 1201, Shimadzu Scientific, Kyoto, Japan, path length of 10 mm at 550 nm) and the direct counting of number of particles in solution with an improved Neubauer counting chamber ( Model SVZ4NIOU, Laboroptik Co., Bad Hamburg, Germany). Pictures were taken with a 3.2 mega pixel digital camera (Pro-MicroScan Model DCM310, Oplenic Co., Hangzhou, China) with an optical microscope (Eclipse model E400, Nikon Instruments, Melville, NY, USA). The images were used to count number of crystals and measure crystal size using the Scope Photo image analysis software (Version 3.0, Oplenic Co., Hangzhou, China). The size of the crystal was taken as the length of a crystal in the **b** direction and the growth rate of the (010) face was measured from the pictures of crystals were taken at the end of experiments.

Ultrasonic Energy (Q) dissipated to the solution was calculated using a calorimetric method according to the equation $Q=(m_{water}\, c_{p\,water} + m_{lactose}\, c_{p\,lactose})(T_f - T_i)$ where m is the weight of solution, $c_p$ is the heat capacity and $T_f$

and $T_i$ are the final and initial solution temperatures (T. J. Mason, Lorimer, & Bates, 1992). Heat capacities of lactose and water are 4.181 and 0.45 kJ $kg^{-1}K^{-1}$ respectively. Power consumed by the ultrasonic probe and mechanical agitator was monitored with a Wattmeter (PC222, ARLEC Electrical, Melbourne, Australia). Power and energy density were expressed as W $g^{-1}$ and J $g^{-1}$. Unless stated, all power densities are power applied, not dissipated. The ultrasonic energy delivered to water and lactose solutions (20-65 g lactose 100 g $water^{-1}$) was also measured using the same method.

Sonication experiments were performed using an ultrasonic horn (Vibracell Model VCX-600, Sonics and Materials Inc., Newtown, CT, USA) with a 13 mm diameter. The solution weight and the position of the probe inside the solution (depth of 30 mm, corresponding to half the length of the probe) were kept constant for all experiments. The device works at a constant frequency of 20 kHz and allows the amplitude to change from 0-100 %, delivering a power range between 55 and 322 W. The reaction vessel was a 600 mL jacketed beaker coupled to a refrigerated recirculator (Model 4850, Bio-Rad Laboratories, Hercules, CA, USA). Since application of ultrasound increases solution temperature, refrigerated water bath temperatures were optimised to keep the solution temperature constant at 22±1°C. Sonication was applied continuously until the absorbance reached 0.1, except at low concentrations (35 and 37.5 g lactose 100 g $water^{-1}$), when stirring experiments continued until abs of 0.05 and a quadratic relationship was used to estimate induction time. Stirring experiments were performed using an overhead stirrer (Model R50D, CAT Co., Staufen, Germany) with a 41 mm flat four blade turbine, in a 500 mL (diameter of 85 mm) glass beaker with four baffles placed in a water bath at 22 °C. Stirring speeds between 200, and 1000 rpm were applied. The maximum stirring speed of the stirrer was 1600 rpm, but above 600 rpm air bubble generation became a significant issue, therefore higher agitation speeds were not applied. Absorbance, Brix and temperature were measured throughout experiments every 1-5 min (Reichert R2 mini handheld digital refractometer, Seefeld, Germany and Digital Thermometer, Model t926 and probe, Model 1293 respectively, Testo Co., Lenzkirch, Germany ).

111 The effect of sonication and stirring on nucleation and growth rate of lactose was investigated under continuous
112 sonication (0.46 W $g^{-1}$) and agitation (300 rpm) at an absolute alpha lactose supersaturation of 14.3 g 100 $g^{-1}$ (60g
113 lactose 100 g $water^{-1}$). Absorbance, crystal number and crystal size were measured as a function of time and were
114 used to determine the critical induction time and the growth rate. Two calibration curves (absorbance versus
115 crystal number) for sonication and stirring were generated. The effect of continuous sonication or stirring on
116 induction time and nucleation rate was investigated at different concentration, ultrasound intensity and agitation
117 speed. The effect of sonication time (15 -900 s) combined with stirring until reaching an absorbance of 0.1 were
118 also investigated at absolute alpha lactose supersaturation of 14.3 g 100 $g^{-1}$. Combination of induction time and
119 energy required to generate same number of crystals allows determining the optimum sonication time.

120 **3. Results and Discussion**

121 **3.1 Delivered energy calculations; power and energy density applied**

122 Power consumption of the sonicator and the agitator at different sonication amplitudes and stirring speeds are
123 given in Table 1. The calorimetric measurements showed that the efficiency of the sonicator was between 20 and
124 45 %. Lactose concentration did not affect the power densities delivered within experimental error.

125 The optimised refrigerated water bath temperatures are given in Table 2. Sonication was initiated at 22 °C as soon
126 as lactose solution was transferred to the jacketed vessel. Temperature was maintained during sonication ±1 °C at
127 applied energy densities of up to 0.73 W $g^{-1}$. Temperature increased by 5 and 10 °C within 9 min of sonication at
128 0.86 and 1.03 W/g power density respectively, showing the limits of the cooling recirculating water bath used. The
129 supersaturation decreased by 7 and 14 % respectively. Therefore the induction times at these ultrasound
130 intensities applied were slightly underestimated.

131 **3.2 The effect of sonication and stirring on crystal number and size**

The effect of sonication and stirring on absorbance, crystal number and size were measured at power density of 0.46 W $g^{-1}$ and 300 rpm stirring speed at an absolute alpha lactose supersaturation of 14.30 g 100 g $water^{-1}$. As can be seen from Figure 1(a), absorbance increased quadratically while crystal number (b) and size (c) increased linearly with time. Sonication resulted in a rapid increase in absorbance compared to stirring. Time taken to reach an absorbance of 0.1 was 7 min with sonication and 44 min with stirring. Sonication resulted in significantly faster nucleation rates than stirring; $5.3x10^5$ and $1.6x10^4$ crystals $mL^{-1}$ $min^{-1}$ respectively from the particle number versus time plot (Figure 1b). On the other hand, the change in the average crystal size (average growth rate) under constant sonication or stirring were found to be the same within experimental error, 0.14 $\mu m$ $min^{-1}$. Formation of secondary nuclei during experiments was unavoidable as sonication or stirring was applied continuously. This resulted in widening of crystal size distribution. The relative standard deviation for sonication was found to be higher than for stirring. This growth rate is in good agreement with the growth rate of lactose crystals given in the literature (Dincer, Ogden, & Parkinson, 2009). The same crystal morphology of a tomahawk was observed for sonicated and agitated crystals.

The rates of nucleation and growth of lactose crystals under sonication have not been reported previously in the literature. Mostly the yield and crystal sizes and amplitude applied (rather than the power or energy density) were reported. Dhumal et al (2008) reported doubling of yield (75-80 %) with a 4-5 times reduction in particle size, together with a change in morphology from a typical tomahawk to rod shaped crystals in an aqueous system using pharmaceutical grade lactose and significantly higher amplitude (75 %, private communication with the author).

Nalajala and Mohalkar (2011) investigated the physical mechanism of sonocrystallisation for a KCl-methanol-water system and reported that the shock waves created by ultrasound affected nucleation, while micro turbulence (micro-convection) governed the growth rate. Crystal growth is a combination of two main steps: firstly, mass transport from solution to the crystal surface by volume diffusion or convection and then secondly, incorporation of growth units into the crystal lattice through surface integration processes (Myerson & Ginde, 2002). The overall

155 growth rate is determined by the slower of these processes. When bulk-phase mass transfer is rate limiting,
156 ultrasonic treatment will enhance the growth rate by increasing the diffusion of growth units to the crystal surface
157 (Ruecroft, Hipkiss, & Naxted, 2005). In the literature, at lactose crystal growth rates below 0.4 and 0.6 $\mu m\ min^{-1}$,
158 the surface integration was reported to be the rate limiting at 30 °C (van Kreveld, 1969); (Dincer , Ogden, &
159 Parkinson, 2009). Hence, at the growth rate measured (0.14 $\mu m\ min^{-1}$) mass transfer rate is not expected to be rate
160 limiting therefore no enhancement of growth rate with ultrasound is expected.

161 **3.3 Estimation of the number of particles in solution and induction time**

162 As can be seen in Figure 2, the relationship between absorbance and particle number is linear for both sonicated
163 and stirred lactose solutions, but sonication has a larger slope than agitation. The absorbance is affected by both
164 crystal number and size. For the sonication experiments, the contribution of size increase to absorbance is
165 negligible as the rate of nucleation is very fast and the duration of experiments is short. In stirred solutions, as the
166 nucleation rates were lower, the duration of experiments were longer, therefore the contribution of growth was
167 higher (Fig 1(c)). The calibration curve generated for stirring takes into consideration the contribution of size
168 increase to absorbance. Additionally, absorbance values less than 0.3 were used to decrease the effect of size on
169 absorbance. Therefore, the change in absorbance was attributed to change in crystal number.

170 The correlation between absorbance and crystal number is: $N_{crystal}$ (# $mL^{-1}$) = Slope of Calibration Curve*abs. Slopes
171 were $2.8x10^6$ and $4.6x10^5$ for sonication and stirring respectively. The only other value reported in the literature is
172 $9\times10^6$ (Mcleod, 2007) for stirring experiments, which is in the same order of magnitude.

173 In this study, the critical induction time was taken as time taken to reach an absorbance of 0.1 although the
174 number of crystals in sonicated and agitated lactose solutions was different. The nucleation rates were calculated
175 by dividing the number of particles at an absorbance of 0.1 by the time taken to reach this value (critical induction
176 time). Counting experiments in section 2.2 allowed comparison of nucleation rates calculated from the direct

177 counting and the critical induction time methods. The difference was found to be ±30 %. Similar relative errors are
178 reported in the literature (Kauter, 2003; Mcleod, 2007).

179 **3.4 The effect of concentration**

180 As can be seen from Figure 3, induction times decreased with increasing supersaturation with both sonicated
181 (dissipated 0.15 W $g^{-1}$) and stirred (at 300 rpm) samples. Ultrasound had a significant effect in reducing induction
182 times. Induction times were, on average, an order of magnitude shorter with sonication compared to stirring which
183 in turn means faster nucleation rates. Application of ultrasound induced significantly faster nucleation at
184 concentrations of approximately 15 g lactose 100 g $water^{-1}$ lower than stirring, which implies that the metastable
185 zone width was narrowed by ultrasound.

186 Nucleation rates increased with increasing concentration for both sonication and stirring (Figure 4). However, the
187 effect of ultrasound was more prominent at low supersaturation (in the intermediate zone, between relative
188 lactose supersaturations of 1.6 and 2.1 (Hourigan, Lifran, Vu , Listiohadi, & Sleigh, 2012)). Similar results were
189 reported in the literature (Li, et al., 2006; Luque de Castro & Priego-Capote, 2007). A plot of $\ln(t_{ind})$ versus $\ln(S)^{-2}$
190 differentiates between homogeneous and heterogeneous nucleation with different slopes (Mullin, 1993) .
191 Homogeneous nucleation involves spontaneous formation of nuclei in the absence of foreign particles and occurs
192 at high concentrations. Existence of foreign particles or surfaces reduces the energy barrier for crystal formation
193 and nucleation occurs at lower supersaturations (Hartel, 2001). Change of mechanisms was observed (Figure 5). In
194 the labile zone (above the supersolubility line), the similar slopes for sonication and stirring indicated that
195 ultrasound did not have any impact on surface energy. In the heterogeneous nucleation zone, the slope of the
196 sonicated is lower than the stirred experiments. A similar result was reported for tolozamide (Kuldipkumar, et al.,
197 2007). At high supersaturation homogeneous nucleation is higher than heterogeneous nucleation, therefore it
198 dominates. At low supersaturation, the rate of homogeneous nucleation is so small that nucleation is mainly
199 heterogeneous nucleation. Application of ultrasound affects heterogeneous nucleation. The decrease in slope

200 with sonication in the heterogeneous nucleation zone is an indication of decreased surface energy which results in
201 decrease in the size of the critical nucleus (Lyczko, Espitalier, Louisnard, & Schwarzentruber, 2002).

202 **3.5 The effect of power**

203 The effect of ultrasound power was investigated at an absolute alpha lactose supersaturation of 14.30 g 100 g
204 water$^{-1}$. As expected, induction times decreased with increasing power (Figure 6). A rapid decline was observed
205 until around 0.46 W g$^{-1}$ applied and then the effect was diminished. At this level, the ultrasonic power density
206 dissipated to the solution was 0.15 W g$^{-1}$ . While the induction time decreased with increasing ultrasound power
207 density, the energy provided to the solution increased. The benefit of reduced induction time with increasing
208 power density therefore needs to be weighed against the increase in energy consumption.

209 In order to compare the power used by sonication and stirring, induction times were plotted as a function of power
210 density (stirring speed 200-1000 rpm, ultrasound power density: 0.15-1.15 W g$^{-1}$ at absolute alpha lactose
211 supersaturation of 14.30 g 100 g water$^{-1}$) Increasing stirring speed up to 600 rpm decreased the induction time, but
212 above this speed the formation and incorporation of large numbers of air bubbles into the solution resulted in an
213 increase in induction time. The maximum nucleation rate achieved was 10,000 # mL$^{-1}$ min$^{-1}$ with agitation. Stirring
214 consumed less energy compared to sonication but it was not possible to achieve the same decrease in induction
215 times as sonication (Figure 7). Increasing agitation speed was reported to reduce induction time up to a certain
216 speed beyond which it remained constant (Myerson & Ginde, 2002). It was also reported that increasing agitation
217 rate diminished the rate of return in lowering the induction time (Mydlarz & Jones, 1991). This is consistent with
218 our findings.

219 **3.6 The effect of sonication time**

220 In the previous sections, sonication was applied continuously to reach an absorbance of 0.1. As sonication is energy
221 intensive and there is no growth rate enhancement, shorter sonication combined with mechanical agitation was

222 investigated. Application of ultrasound is expected to result in a larger number of crystals in solution in significantly
223 shorter periods of time compared to agitation and it might be expected to increase the rate of secondary
224 nucleation created by agitation as well as heterogeneous nucleation.

225 In these experiments, 60 g 100 $g^{-1}$ lactose solutions were sonicated at 0.47 W $g^{-1}$ power density from 15 to 900 s
226 followed by stirring at 300 rpm at 22°C . Total energy used to reach an abs of 0.1, which is equivalent to generation
227 of $3X10^6$ particle $mL^{-1}$, termed the critical number, was calculated by incorporating both sonication and stirring
228 components. Sonication decreased the time to reach the critical number significantly (Figure 8). Even 15 s
229 sonication reduced the time and energy compared to stirring only. Beyond 3 min, no benefits of further sonication
230 were observed. The minimum energy required to reach the critical number was approximately 75 J $g^{-1}$, which was
231 reached after around 1 min sonication. Beyond this point, the energy required increased linearly with sonication
232 time. The minimum energy point will vary with concentration and ultrasound power intensity.

233 **4. Conclusions**

234 Lactose crystal morphology and growth rates were found not to be affected by ultrasound under the experimental
235 conditions investigated but induction times were reduced and nucleation rates were increased significantly with
236 the application of ultrasound. Sonication resulted in significantly faster nucleation than stirring. Power input for
237 sonication was much higher than for the mechanical agitator but the nucleation rate achieved by sonication was
238 significantly faster. The fastest possible stirring rate applied in these experiments resulted in much slower
239 nucleation than the lowest possible power density with the sonicator. Induction time decreased rapidly until the
240 delivered power intensity was 0.15 W $g^{-1}$. Even 15 s sonication introduces more nucleation than stirring. The
241 sonication times up to 3 min decreased induction time with only minor reductions after this time. One min
242 sonication was found to be the optimum sonication time under the experimental conditions.

Application of ultrasound has the potential to introduce a large number of nuclei in a shorter period of time compared to mechanical agitation, which will result in increased secondary nucleation and higher yields. Under the experimental conditions tested, the minimum energy required to reach the critical number was approximately 75 J $g^{-1}$. This is quite large considering the volumes of whey processed in the industry. On the other hand, nucleation rates in whey and permeate were reported to be 5 to 10 times faster than in aqueous solutions (Mcleod, 2007) and it is likely that shorter sonication times would be needed for industrial lactose crystallisation from whey or permeate, compared to pure water. The effect of ultrasound on lactose crystallisation needs to be investigated in concentrated whey to assess the potential of ultrasound to be implemented in industrial lactose crystallisation.

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## References

Bund, R., & Pandit, A. (2007). Sonocrystallisation: Effect on lactose recovery and crystal habit. *Ultrasonics Sonochemistry, 14*, 143-152.

Butler, B. (1998). *Modelling Industrial Lactose Crystallisation.* Ph D University of Queensland, Brisbane, Australia.

Dhumal, R., Biradar, S., Paradkar, A., & York, P. (2008). Ultrasound assited engineering of lactose crystals. *Pharmaceutical Research, 25*(12), 2835-2843.

Dincer , T. D., Ogden, M. I., & Parkinson, G. M. (2009). Crystal growth mechanisms of the (010) face of alpha lactose monohydrate crystals. *Journal of Crystal Growth, 311*, 2427-2432.

Dincer, T. D., Ogden, M. I., & Parkinson, G. M. (2009). In situ investigation of growth rates and growth rate dispersion of $\alpha$-lactose monohydrate. *Journal of Crystal Growth, 311*, 1352-1358.

Gherras, N., & Fevotte, G. (2012). Comparison between approaches for the experimental determination of metastable zone width: a case study of the batch cooling crystallization of ammonium oxalate in water. *Journal of Crystal Growth 342*(1), 88-98.

Hartel, R. W. (2001). *Crystallisation in Foods*. Gaithersburg, Maryland, USA: An Aspen Publication.

Hourigan, J. A., Lifran, E. V., Vu , L. T. T., Listiohadi, Y., & Sleigh, R. W. (2012). Lactose: Chemistry, Processing and Utilisation. In G. W. S. M. A. Augustin (Ed.), *Advances in Dairy Ingredients* (pp. 31-69). Oxford: Wiley-Blackwell & IFT Press.

Kauter, M. (2003). *The effect of impurities on lactose crystallisation.* PhD, University of Queensland, Brisbane, Australia. (146)

Kobari, M., Kubota, N., & Hirasawa, I. (2012). Secondary nucleation-mediated effects of stirrer speed and growth rate on induction time for unseeded solution. *Crystal Engineering Communications, 14*, 5255-5261.

Kougoulos, E., Marziano, I., & Miller, P. R. (2010). Lactose particle engineering: Influence of ultrasound and anti-solvent on crystal growth and habit. *Journal of Crystal Growth, 312*, 3509-3520.

276 Kuldipkumar, A., Kwon, G. S., & Zhang, G. G. Z. (2007). Determination of growth mechanism of tolazamide by induction time
277 measurement. *Crystal Growth and Design, 7*(2), 234-242.
278 Li, H., Li, H., Guo, Z., & Liu, Y. (2006). The application of power ultrasound to reaction crystallisation. *Ultrasonics*
279 *Sonochemistry, 13*, 359-363.
280 Luque de Castro, M., & Priego-Capote, F. (2007). Ultrasound-assited crystallisation (sonocrystallisation). *Ultrasonics*
281 *Sonochemistry, 14*, 717-724.
282 Lyczko, N., Espitalier, F., Louisnard, O., & Schwarzentruber, J. (2002). Effect of ultrasound on the induscton time and the
283 metasrable zone widths of potassium sulphate. *Chemical Engineering Journal (Lausanne), 86*, 233-241.
284 Mason, T., & Lorimer, J. (2002). *Applied Sonochemistry*. Weinhiem: Wiley-VCH.
285 Mason, T. J., Lorimer, J. P., & Bates, D. M. (1992). Quantifying sonochemistry: casting some light on black art. *Ultrasonics*
286 *Sonochemistry, 30*(1), 40-42.
287 Mcleod, J. (2007). *Nucleation and Growth of Alpha Lactose Monohydrate.* PhD PhD, Massey University, Palmerston North,
288 New Zealand. Retrieved from http://mro.massey.ac.nz/handle/10179/1444
289 Mullin, J. W. (1993). *Crystallization* (3rd ed. Vol. Chapter 5). Oxford: Butterworth-Heinemann Ltd.
290 Mydlarz, J., & Jones, A. G. (1991). Crystallisation and agglomeration kinetics during the batch drowning out precipitation of
291 potash alum with aqueaus acetone. *Powder Technology, 65*, 187-194.
292 Myerson, A. S., & Ginde, R. (2002). Crystals, Crystal Growth and Nucleation. In A. S. Myerson (Ed.), *Handbook of Industrial*
293 *Crystallisation* (Second Edition ed.). Walthan, MA, USA: Butterworth Heinemann.
294 Nalajala, V., & Moholkar, V. (2011). Investigation in the physical mechanisms of sonocrystallisation. *Ultrasonics*
295 *Sonochemistry, 18*, 345-355.
296 Patel, S., & Murthy, Z. (2009). Ultrasound assited crystallisation for the recovery of lactose in an anti-solvent acetone. *Crystal*
297 *Research and Technology, 44*(8), 889-896.
298 Ruecroft, G., Hipkiss, D., & Naxted, T. (2005). Sonocrystallisation: the use of ultrasound for improved industrial crystallisation.
299 *Organic Process Research & Development, 9*, 923-932.
300 van Kreveld, A. (1969). Growth rates of lactose crystals in solutions of stable anhydous alpha lactose. *Netherlands Milk and*
301 *Dairy Journal, 23*, 158-275.
302 Visser, R. A. (1982). Supersaturation of alpha lactose in aqueous solutions in mutarotation equilibrium. *Netherlands Milk and*
303 *Dairy Journal, 36*, 89-101.
304 Westergaard, V. (2010). Milk Powder Technology Evaporation and Spray Drying Retrieved from http://www.niro.com/milk-
305 powder-technology/milk-powder-technology.pdf
306 Zisu, B., Bhaskaracharya, R., Kentish, S., & Ashokkumar, M. (2010). Ultrasonic processing of dairy systems in large reactors.
307 *Ultrasonics Sonochemistry, 17*, 1075-1081.

308

309 **List of Figures**

310 **Fig. 1.** Change in (a) absorbance (b) particle number and (c) crystal size as a function of time for continuous

311 sonication (0.46 W $g^{-1}$) (◆) and stirring (300 rpm) (■) for lactose solutions at an absolute alpha lactose

312 supersaturation of 14.30 g $100g^{-1}$. Error bars are standard deviation of two measurements in the particle counting

313 and on average 100 crystal size measurements in crystal size measurement.

314

**Fig. 2.** Plot of absorbance versus particle number for continuous sonication (0.46 W $g^{-1}$) (◆) and stirring (300 rpm) (■) for lactose solutions at an absolute alpha lactose supersaturation of 14.30 g $100g^{-1}$. Error bars are standard deviation of two measurements.

**Fig. 3.** The critical induction time for continuous sonication (0.46 W $g^{-1}$) (◆) and stirring (300 rpm) (■) as a function of an absolute alpha lactose supersaturation. Secondary nucleation threshold (....) and Supersolubility (- - -) at 22 °C.

**Fig. 4. Plot of** nucleation rate for continuous sonication (0.46 W $g^{-1}$) (◆) and stirring (300 rpm) (■) as a function of absolute alpha lactose supersaturation. Secondary nucleation threshold (....) and Supersolubility (- - -) at 22 °C. Error bars are 30% as calculated in Section 3.4

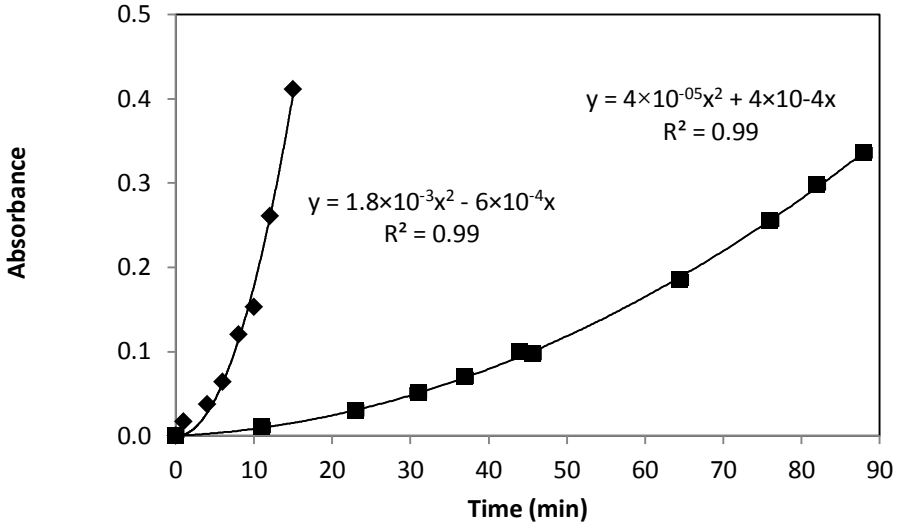
**Fig. 5.** Plot of $\ln(t_{ind})$ versus $\ln^{-2}$ (S) for lactose crystallisation in a continuous sonication (0.46 W $g^{-1}$) (◆) and stirring (300 rpm, 0.03 W $g^{-1}$) (■). Supersolubility at 22 °C (- - -).

**Fig. 6.** The plot of induction time versus of applied ultrasonic power density for lactose solution at an absolute alpha lactose supersaturation of 14.30 g $100g^{-1}$ with continuous sonication. Error bars are standard deviation of duplicate experiments.

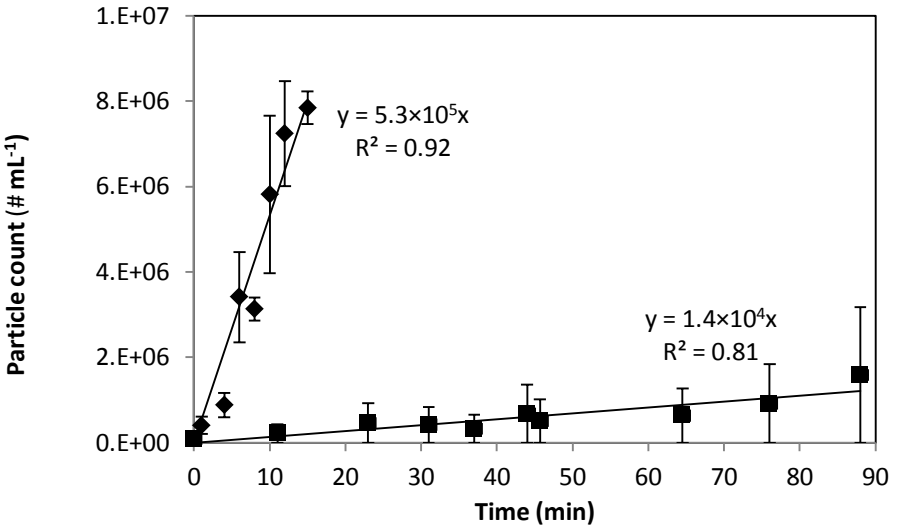
**Fig. 7.** The nucleation rates of lactose solutions at an absolute alpha lactose supersaturation of 14.30 g $100g^{-1}$ for continuous sonication (◆) and stirring (■) as a function of applied power density. Sonication was applied at 2-90% amplitudes which corresponds to 0.15- 1.15 W $g^{-1}$ and stirring between 200-1000 rpm which corresponds to 0.02-0.08 W $g^{-1}$.

**Fig. 8**. The total time and energy required to generate $2.8\times10^{6}$ nuclei per ml of solution as a function of sonication time. Continuous sonication (0.46 W $g^{-1}$) was applied followed by stirring at 300 rpm at an absolute alpha lactose supersaturation of 14.30 g 100 $g^{-1}$.

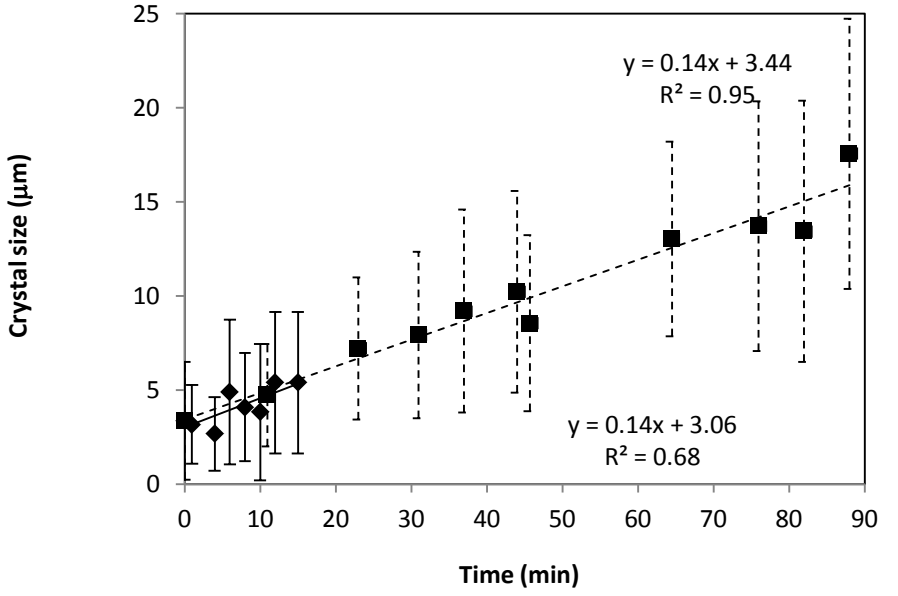
(a)

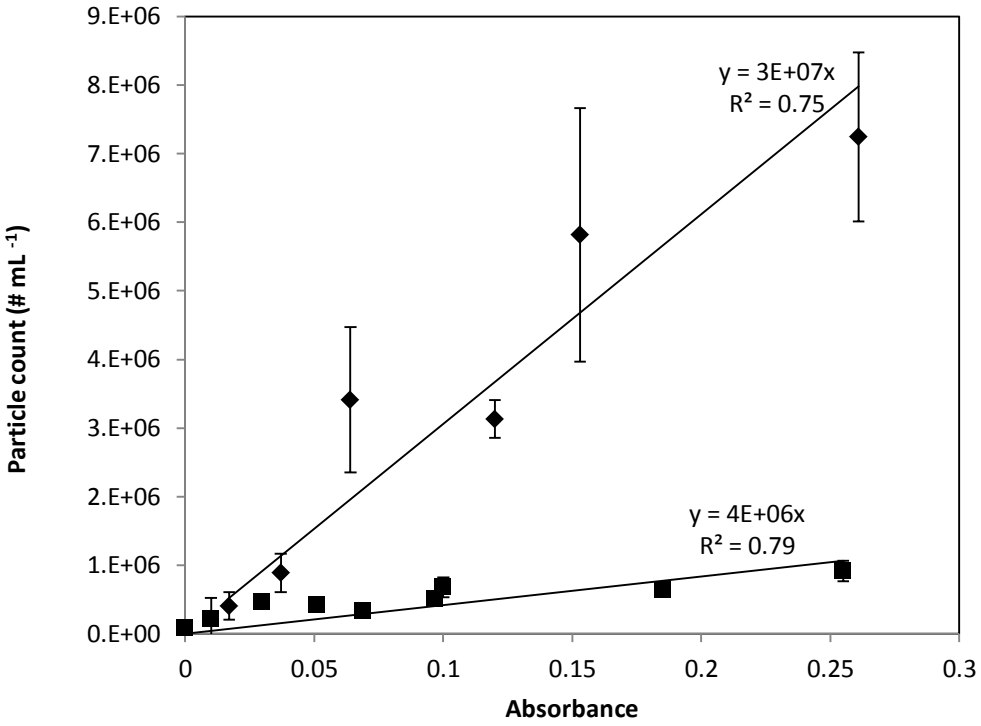

Absorbance
Time (min)
y = 4×10-05x2 + 4×10-4x
R² = 0.99
y = 1.8×10-3x2 - 6×10-4x
R² = 0.99

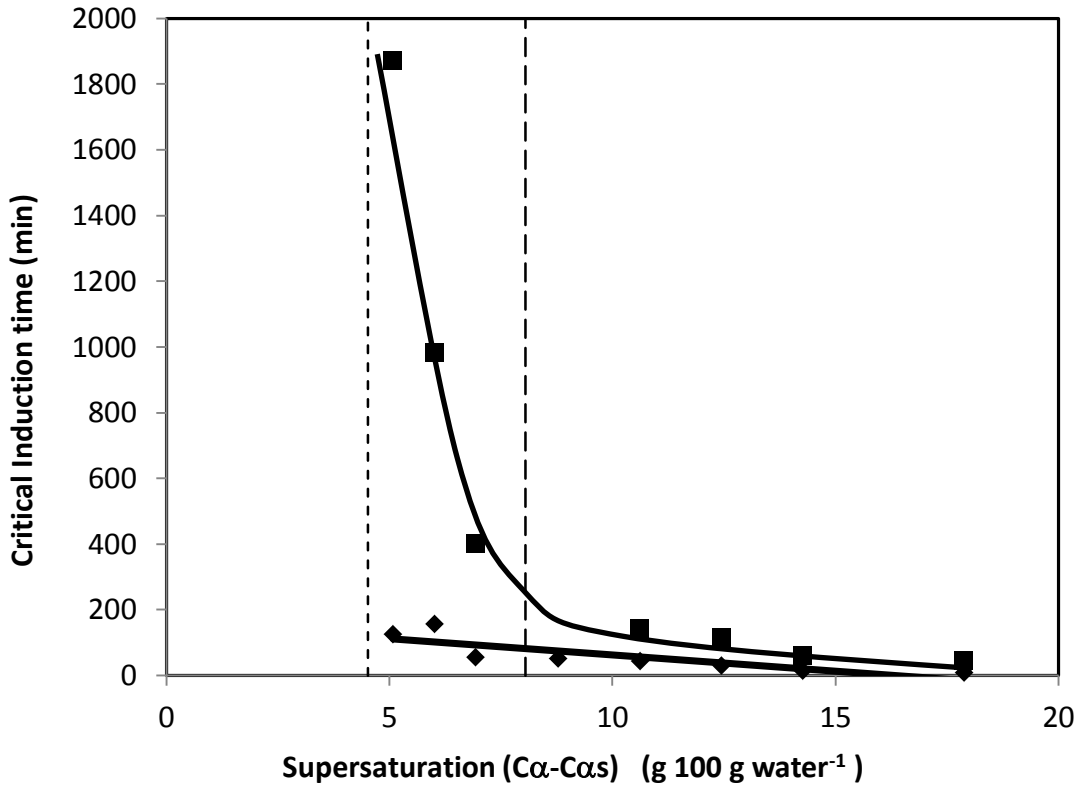
(b)

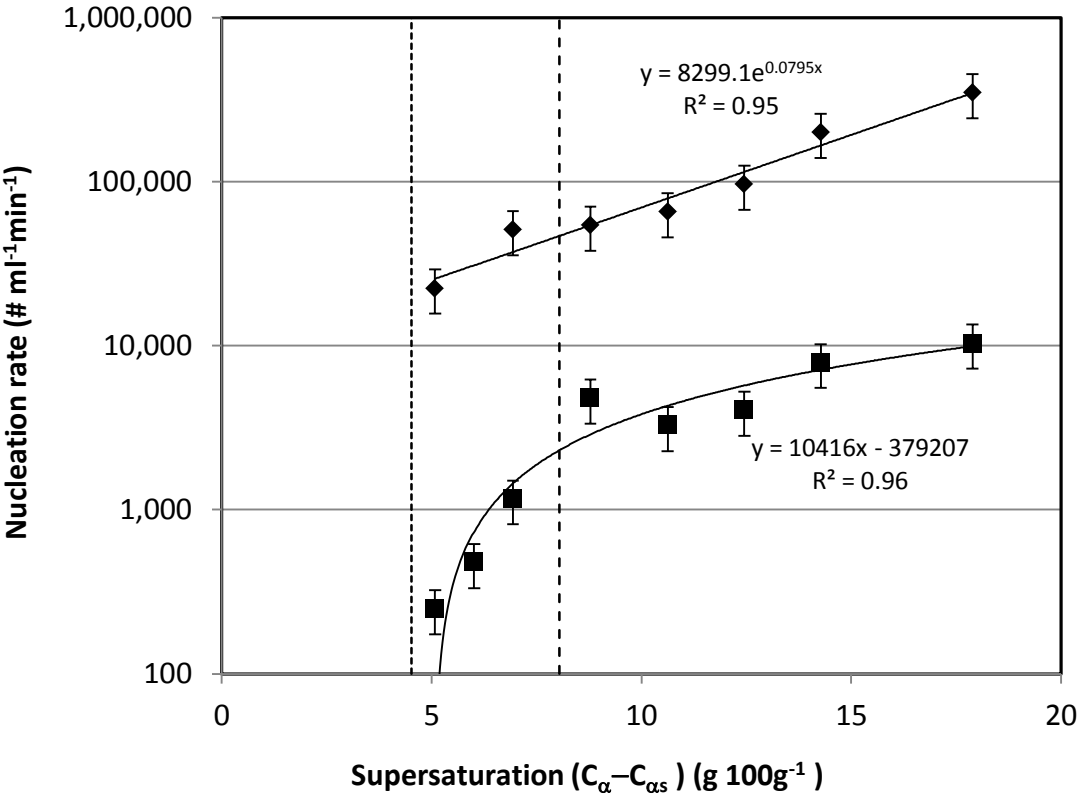

Particle count (# mL-1)
Time (min)
y = 5.3×10⁵x
R² = 0.92
y = 1.4×10⁴x
R² = 0.81

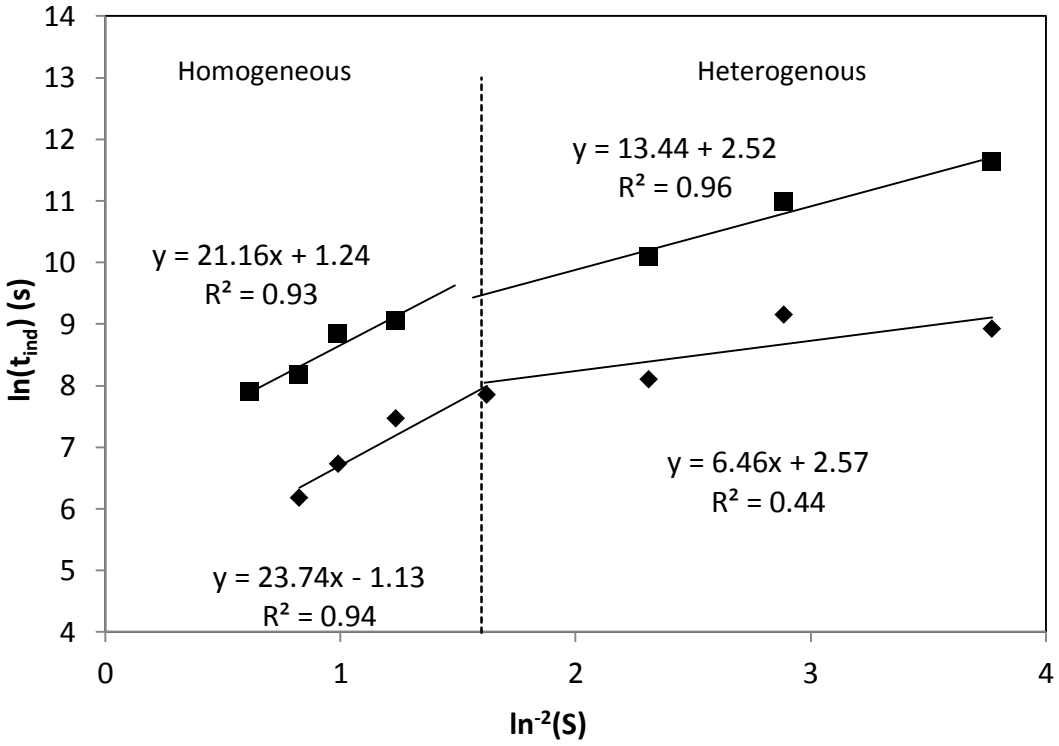
(c)

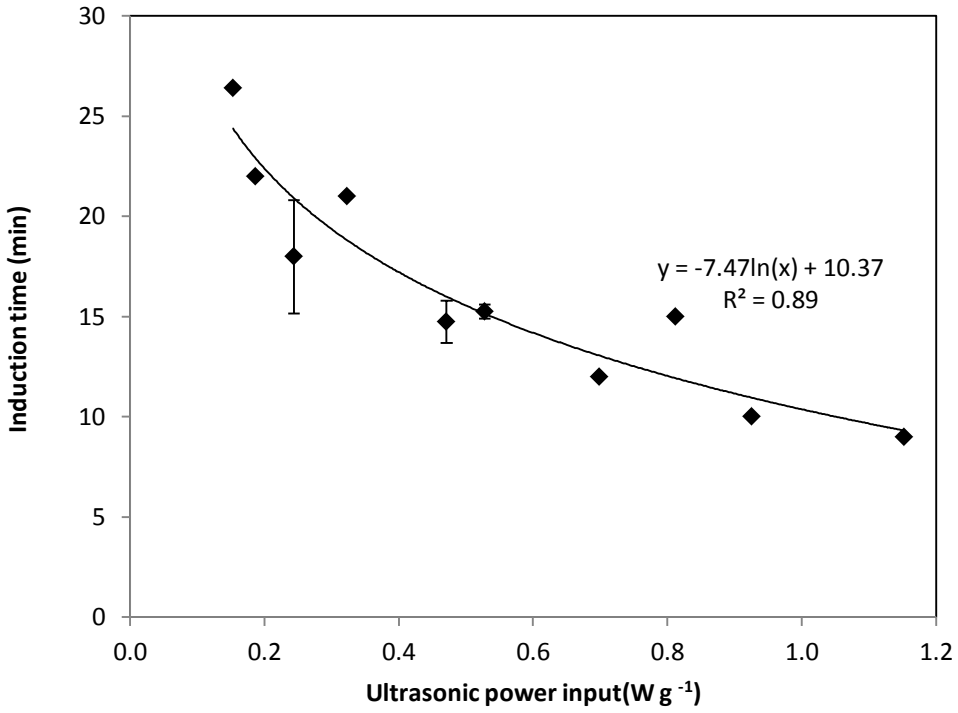

Crystal size (μm)
Time (min)
y = 0.14x + 3.44
R² = 0.95
y = 0.14x + 3.06
R² = 0.68

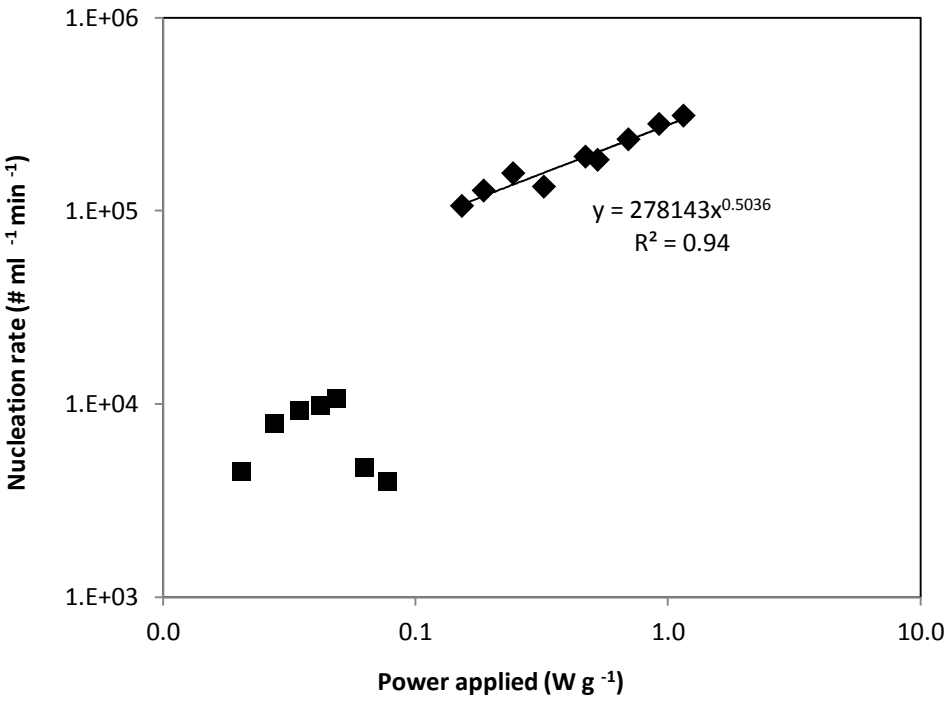

9.E+06
8.E+06
7.E+06
6.E+06
5.E+06
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
Particle count (# mL -1)
y = 3E+07x
R² = 0.75
y = 4E+06x
R² = 0.79
0
0.05
0.1
0.15
0.2
0.25
0.3
Absorbance


2000
1800
1600
1400
1200
1000
800
600
400
200
0
Critical Induction time (min)
0
5
10
15
20
Supersaturation (Cα-Cαs) (g 100 g water-1 )


1,000,000
100,000
10,000
1,000
100
0
5
10
15
20
Nucleation rate (# ml-1min-1)
Supersaturation (Cα–Cαs ) (g 100g-1 )
y = 8299.1e0.0795x
R² = 0.95
y = 10416x - 379207
R² = 0.96


Homogeneous
Heterogenous
y = 13.44 + 2.52
R² = 0.96
y = 21.16x + 1.24
R² = 0.93
y = 6.46x + 2.57
R² = 0.44
y = 23.74x - 1.13
R² = 0.94
ln(t_ind) (s)
ln^-2(S)
14
13
12
11
10
9
8
7
6
5
4
0
1
2
3
4


30
25
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Induction time (min)
Ultrasonic power input(W g -1)
y = -7.47ln(x) + 10.37
R² = 0.89


1.E+06
1.E+05
1.E+04
1.E+03
Nucleation rate (# ml -1 min -1)
y = 278143x0.5036
R² = 0.94
0.0
0.1
1.0
10.0
Power applied (W g -1)

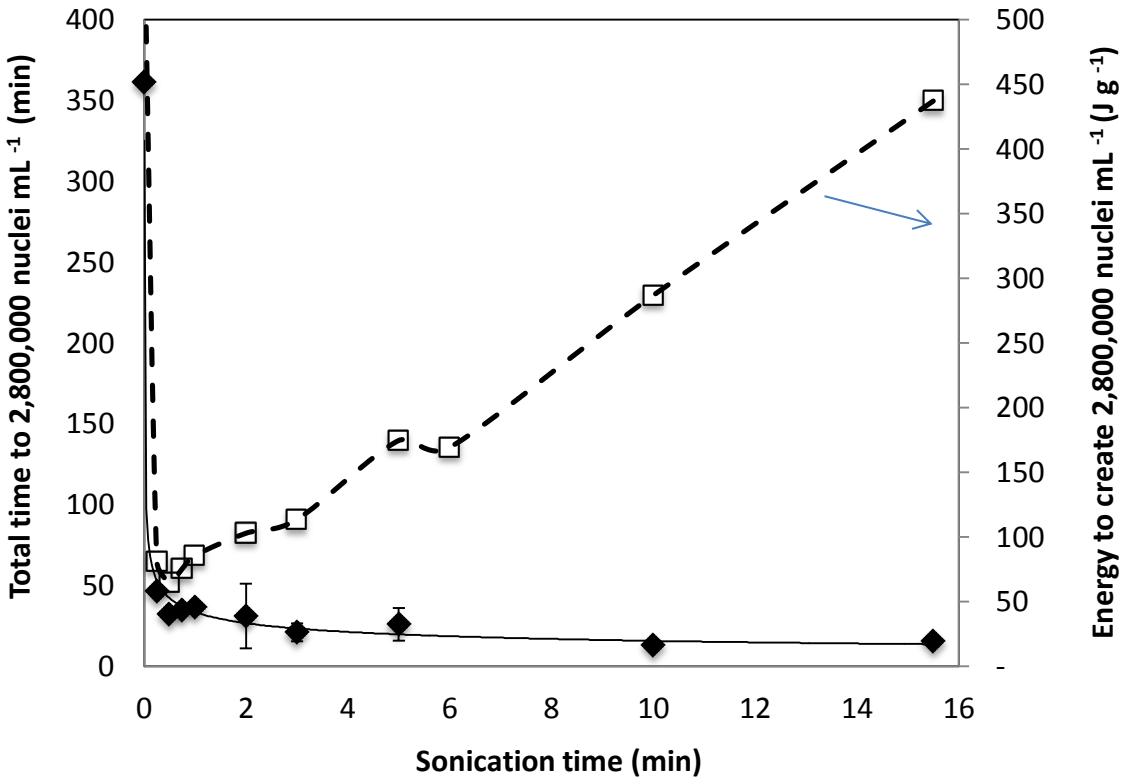

Total time to 2,800,000 nuclei mL -1 (min)
Energy to create 2,800,000 nuclei mL -1 (J g -1)
Sonication time (min)
400
350
300
250
200
150
100
50
0
500
450
400
350
300
250
200
150
100
50
-
0
2
4
6
8
10
12
14
16

**Table 1**

Power used by the sonicator and the mechanical stirrer as a function of amplitude or rpm tested.

| Sonicator | | | Stirrer | |
|---|---|---|---|---|
| **Amplitude (%)** | **Power applied (W)** | **Power delivered (W)** | **Stirrer speed (rpm)** | **Power applied (W)** |
| 0 | 55 | 0 | 200 | 6 |
| 10 | 90 | 10 | 300 | 8 |
| 20 | 108 | 31 | 400 | 10 |
| 40 | 162 | 52 | 500 | 13 |
| 60 | 218 | 84 | 600 | 15 |
| 80 | 282 | 126 | 800 | 19 |
| 100 | 322 | 147 | 1000 | 23 |

**Table 1**
Optimised water bath temperature set points as a function of amplitude for an absolute alpha lactose supersaturation of 14.30 g 100 g water$^{-1}$

| Amplitude (%) | Set point (°C) |
|---|---|
| 2 | 22.5 |
| 10 | 20.0 |
| 12 | 19.0 |
| 17 | 16.5 |
| 25 | 13.0 |
| 30 | 11.0 |
| 35 | 7.5 |
| 50 | 0.0 |
| 60 | -3.5 |