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1 **The emerging application of ultrasound in lactose crystallisation**

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8 **Abstract:** Ultrasonic processing is the industrial application of sound waves with a frequency
9 above the upper limit of human hearing. Interest has arisen recently in the effects of
10 ultrasound on the crystallisation of lactose as an innovative technology to improve its
11 recovery and the control over its crystal properties. This not only will increase the financial
12 profit for lactose manufacturers and improve the quality of lactose for specific applications,
13 but will also improve the quality of end products manufactured with lactose as an ingredient.

14 **Short title:** Ultrasonic crystallisation of lactose

15 **Keywords:** Lactose, crystallisation, ultrasound, recovery, particle engineering

16 **Abbreviations:** 7-ACDA: 7-amino-3-desacetoxy cephalosporanic acid; CSD: crystal size
17 distribution; DPI: dry powder inhaler; MSZW: metastable zone width

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23 **1. Introduction**

24 The first reports suggesting the ability of ultrasound to induce physical and chemical changes
25 in materials were published in the late 1920s (Leonelli & Mason, 2010; Richards, 1929;
26 Richards & Loomis, 1927; Wood & Loomis, 1927). However, the use of ultrasound started to
27 grow only after the 1970s due to the more general availability of commercial ultrasonic
28 equipment (Leonelli & Mason, 2010). The initial industrial applications of power ultrasound
29 were in cleaning and plastic welding, which still continue to be the most popular applications
30 (Mason, 2003). Due to the rapid growth of the technology in recent years, ultrasound has
31 become a viable alternative option for some conventional food processing methods, such as
32 emulsification, homogenisation and extraction (Ashokkumar et al., 2008; Patist & Bates,
33 2008). Ultrasound has also been shown to improve other traditional processes such as
34 filtration, extraction and crystallisation (Patist & Bates, 2008).

35 Ultrasonic processing is the application of sound waves in the frequency range over 20 kHz,
36 which is above human hearing (Leonelli & Mason, 2010; Patel & Murthy, 2009). Ultrasound
37 has been categorised into ‘high-intensity or power ultrasound’ (20 - 100 kHz) (Suzuki, Lee,
38 Padilla, & Martini, 2010), which has usages in food processing, ‘high-frequency ultrasound’
39 (100 kHz - 1 MHz) and ‘diagnostic ultrasound’ (1 - 10 MHz), which has medical applications
40 (Patist & Bates, 2008). However, the majority of food processing studies have been limited to
41 the range of 20 - 40 kHz (Mason, 1998), due to the higher physical effects (cavitation) and
42 insignificant chemical effects (radical production) at lower frequencies (Ashokkumar et al.,
43 2010; Hem, 1967).

44 Most studies on ultrasonic crystallisation have focused on its use in the manufacture of fine
45 chemicals and pharmaceuticals, in attempts to increase their compliance with strict standards
46 (Ruecroft, Hipkiss, Ly, Maxted, & Cains, 2005). Ultrasonic crystallisation is an under-
47 researched area in food technology (Deora, Misra, Deswal, Mishra, Cullen, & Tiwari, 2013),
48 with only a small number of reports on its use in the crystallisation of food materials such as
49 milk fat (Suzuki et al., 2010), sunflower oil (Arends, Blindt, Janssen, & Patrick, 2003), ice
50 (Chow, Blindt, Chivers, & Povey, 2003) and lactose (Zamanipoor, Dincer, Zisu, & Jayasena,
51 2013).

52 Interest has arisen in the last few years in the study of the effects of ultrasound on the
53 crystallisation of lactose, particularly its potential ability to induce desirable crystal properties
54 and to improve lactose recovery (Bund & Pandit, 2007b; Bund & Pandit, 2007c; Dhumal,
55 Biradar, Paradkar, & York, 2008; Kougoulos, Marziano, & Miller, 2010; Patel & Murthy,
56 2009; Patel & Murthy, 2010; Patel & Murthy, 2011a; Patel & Murthy, 2011b; Zamanipoor et
57 al., 2013). This review paper aims to critically discuss this emerging technique to provide an
58 overall perspective of the benefits of the application of ultrasound in lactose crystallisation.

59 **2. The process of crystallisation**

60 The process of crystallisation from a solution has three distinct phases: formation of
61 supersaturation (due to the difference between the solute concentration and solubility),
62 nucleation (appearance of crystals) and crystal growth (Brito & Giulietti, 2007; Bund &
63 Pandit, 2007a).

64 **2.1. Formation of supersaturation**

65 At any given temperature, a maximum quantity of solute can be dissolved in a solvent (Brito
66 & Giulietti, 2007). When a solution is saturated with a solute, it is considered to be in a
67 thermodynamic equilibrium. However, an increase in concentration above the saturation
68 (solubility) point disrupts the equilibrium, forming supersaturation and under these conditions
69 crystallisation may occur (Deora et al., 2013).

70 In a plot of the temperature versus solute concentration, the region between the solubility and
71 super-solubility curves is called the metastable zone width (MSZW), as shown for lactose in
72 Fig. 1. In this region, despite the presence of supersaturation, crystallisation does not occur
73 spontaneously (Shi, Hartel, & Liang, 1989; Wong, Bund, Connelly, & Hartel, 2011b). The
74 time elapsed between the formation of supersaturation and the spontaneous appearance of
75 crystals is referred to as 'induction time'. The induction time is a function of supersaturation
76 and decreases with an increase in supersaturation (Luque de Castro & Priego-Capote, 2007;
77 Patel & Murthy, 2009).

78 Long induction time and wide MSZW are the factors responsible for the slow crystallisation
79 of lactose, which necessitates very high supersaturation to induce nucleation (Dhumal et al.,
80 2008; Patel & Murthy, 2009; Patel & Murthy, 2012; Raghavan, Ristic, Sheen, & Sherwood,
81 2001). Seed addition and the use of anti-solvents have been used to try to reduce the
82 induction time and accelerate the crystallisation of lactose (Dhumal et al., 2008).

83 **2.2. Nucleation and growth**

84 Nucleation involves the initial formation of crystals in a supersaturated solution (Brito &
85 Giulietti, 2007). It is an activated process in which the transition state is associated with the
86 binding of molecules through intermolecular forces, such as hydrogen bonds, π - π and *van der*
87 *Waals* interactions (McLeod, 2007). As shown in Fig. 2, nucleation can be induced in two
88 different pathways: (1) spontaneous nucleation, which can only happen at very high levels of
89 supersaturation in the labile zone (primary, homogeneous nucleation), and (2) nucleation
90 induced by a solid interphase (such as a container wall or a pre-existing particle, in which
91 case it is called primary, heterogeneous nucleation; or a crystal of the solute, in which case it
92 is called secondary nucleation). Secondary nucleation occurs because the crystals of the
93 solute can either act as templates for the formation of new nuclei or break up to form further
94 new nuclei (Luque de Castro & Priego-Capote, 2007).

95 Once the nuclei are formed, they grow into large crystals in a growth process (Rodríguez-
96 Hornedo & Murphy, 1999). The following mechanisms are generally assumed to be involved
97 in crystal growth: bulk diffusion, surface diffusion and integration of growth units into the
98 crystal surface. The growth rate is determined by the growth-limiting step, which is the
99 slowest of the above mechanisms (Farhadi & Babaheidary, 2002; McLeod, 2007). For α -
100 lactose, surface integration is reported to be the rate-limiting step (Thurlby, 1976).

101 Supersaturation is the driving force for nucleation and growth (Visser, 1982). Spontaneous
102 primary nucleation cannot occur inside the MSZW since the energy available in the
103 supersaturation in this region is not adequate to induce nuclei formation (Shi et al., 1989).
104 However, it is possible to induce nucleation in the upper region of the MSZW (the area
105 between the forced crystallisation and supersolubility curves in Fig. 1) using seeding or
106 forced nucleation (Shi et al., 1989; Wong et al., 2011b).

107 Both nucleation and the growth of crystals are system specific and highly dependent on
108 supersaturation. Hence, increased control over nucleation and the growth of crystals and
109 consequently on crystal properties can be achieved by controlling the supersaturation.
110 Although both nucleation and the crystal growth occur rapidly at higher supersaturations in
111 the labile zone, it is generally desirable not to increase the supersaturation to such high levels.
112 Operating in this region, while promoting crystal growth, causes uncontrollable nucleation
113 leading to a low mean crystal size and a wide crystal size distribution (CSD) (Patel &
114 Murthy, 2009; Patil, Gore, & Pandit, 2008; Paul, Tung, & Midler, 2005). A wide CSD is
115 undesirable due to the resulting difficulties in processing such as in centrifugation, filtration
116 and washing, leading to reduced recovery and poor final product quality (Shi, Liang, &
117 Hartel, 2006).

118 In order to achieve large particles with minimal CSD it is necessary to maximise growth and
119 minimise secondary nucleation (Wong et al., 2011b). A growth-dominated process also has
120 other advantages, such as the formation of crystals with lower surface area (easier to wash
121 and dry, with lower entrapment of mother liquor), higher bulk density (easier to pack) and
122 reduced formation of agglomerates (Paul et al., 2005). Paul et al. (2005) suggested that, in
123 order for crystal growth to dominate over nucleation, it is necessary to control and limit the
124 supersaturation. Since nucleation is more energy-demanding than growth (Rodríguez-
125 Hornedo & Murphy, 1999), it can be assumed that under limited supersaturation, i.e. within
126 the MSZW, crystal growth will dominate. Consequently, in order to achieve large crystals
127 with minimal CSD, it has been proposed to operate the process of crystallisation inside the
128 MSZW and use seeding to induce nucleation (Wong, Bund, Connelly, & Hartel, 2011a;
129 Wong et al., 2011b). On the other hand, in order to achieve reliable crystallisation
130 performance, it has been suggested that operation should be carried out in the labile zone
131 away from the MSZW (O'Grady, Barrett, Casey, & Glennon, 2007). Consequently, it can be
132 inferred that in conventional crystallisation, the conditions that enable control over the
133 crystallisation process and crystal properties (i.e. operating at low supersaturation levels
134 inside the MSZW) cannot offer the maximum process efficiency and performance.

135 **3. Ultrasonic crystallisation**

136 Ultrasonic crystallisation is the use of power ultrasound which is mainly applied during the
137 nucleation phase to control the crystallisation process (Bund & Pandit, 2007c; Deora et al.,
138 2013).

139 Ultrasound is transmitted as a series of compression and rarefaction cycles. Rarefaction
140 cycles increase molecular distances by overcoming the attractive forces between molecules in
141 the liquid and form cavities which continue to grow in size to create cavitation bubbles
142 (Leonelli & Mason, 2010). The bubbles then disperse throughout the liquid in the form of
143 filament patterns due to the interaction forces with the sound field and other bubbles
144 (Lauterborn, Kurz, Geisler, Schanz, & Lindau, 2007). Many thousands of cavitation bubbles
145 are generated, some of which are rather stable (stable cavitation), but most others grow into
146 an unstable size at which point they collapse violently (transient cavitation), releasing a
147 powerful shockwave (Deora et al., 2013; Leonelli & Mason, 2010) (Fig. 3).

148 Three mechanisms have been proposed in the literature which supposedly promote nucleation
149 in a crystallisation process as affected by ultrasound: (1) the shockwave released from
150 cavitation bubbles promotes mass transfer and molecular collisions leading to the formation
151 of primary nuclei (Cains, Martin, & Price, 1998; Dhumal et al., 2008; Guo, Jones, & Li,
152 2006b; Guo, Zhang, Li, Wang, & Kougoulos, 2005; Luque de Castro & Priego-Capote, 2007;
153 Patel & Murthy, 2009), (2) the aeration caused by the formation and movement of cavitation
154 bubbles promotes mass transfer (Li, Li, Guo, & Liu, 2006), and (3) the evaporation from the
155 internal surface of the bubbles results in localised cooling, leading to the development of very
156 high internal supersaturation and nucleation, which enables the bubbles to act as nucleation
157 centres (Hem, 1967). Although these mechanisms generally agree that ultrasound promotes
158 nucleation, there is no consensus regarding the effect of ultrasound on crystal growth. Some
159 reports have indicated that ultrasound promotes crystal growth (Li et al., 2006; Mason, 1998),
160 while others have reported obtaining smaller crystals by sonication and/or highlighted the
161 effect of ultrasound on the disruption of grown crystals (Chow et al., 2003; Hem, 1967; Patel
162 & Murthy, 2009; Patel & Murthy, 2011a; Suzuki et al., 2010).

163 Guo et al. (2005) and Li et al. (2006) studied the effect of ultrasound on the induction times
164 of roxithromycin and 7-amino-3-desacetoxy cephalosporanic acid (7-ACDA), respectively,
165 and reported that the induction time of the sonicated sample was significantly shorter than the
166 control, especially at lower supersaturations. In other words, the induction time of the
167 sonicated sample at lower concentrations was shorter than that for the non-sonicated sample
168 at higher concentrations. They concluded that sonication can narrow the MSZW and induce
169 nucleation at lower supersaturations than conventional mixing. This reduction in induction
170 time has been ascribed to an acceleration in diffusion induced by the ultrasonic power,
171 suggesting a diffusion-controlled mechanism (Guo et al., 2005).

172 Guo et al. (2005) also reported a reduction in the crystal size of roxithromycin induced by
173 ultrasound and correlated it to the disrupting effect of shockwaves and abrasion between
174 crystals, leading to the formation of smaller crystals. On the other hand, Li et al. (2006)
175 reported obtaining larger 7-ACDA crystals using ultrasound and correlated it to the rapid
176 growth and improved mass transfer induced by sonication.

177 Dalas (2001) reported no change in the typical rhombohedral morphology of calcite crystals
178 and the spherical morphology of vaterite crystals as affected by ultrasound, and concluded
179 that ultrasound did not result in the preferential growth of a certain crystal face. However,
180 Guo et al. (2005) observed that ultrasound induced a change in the crystal shape of
181 roxithromycin from hexagonal to rhombus, and Amara, Ratsimba, Wilhelm and Delmas
182 (2001) noticed a change in the crystal shape of potash alum from octahedral to decahedral.
183 They correlated the change in the typical crystal shape mainly to the fact that ultrasound can
184 preferentially affect the growth rate of certain crystal faces.

185 Table 1 summarises the different reported effects of ultrasound on the crystallisation of a
186 variety of chemical compounds. It is apparent that contrasting effects have been reported for
187 different chemical compounds, and sometimes for the same chemical compound but under
188 different experimental conditions. These contrasting findings highlight that the effects of
189 ultrasound on a crystallisation system depend on the nature of the system. Hence, it is of the
190 utmost importance that the behaviour of each crystallisation system as affected by ultrasound
191 be investigated individually. Adjusting the ultrasonic variables to achieve desirable
192 crystallisation effects will also depend on the findings of such investigations for each system.

193 **4. Lactose and its crystallisation**

194 Lactose is the major carbohydrate in milk. It is a disaccharide composed of galactose and
195 glucose linked by a β (1 \rightarrow 4) glycosidic bond (Fox, 2009; Wong et al., 2011b). It is present in
196 the milk of all mammals (with only a few exceptions) at an approximate concentration of 2 –
197 10% by weight, and can be found in bovine milk at an average concentration of 4.8%.

198 Lactose has a level of sweetness about 20% that of sucrose. The crystals of α -lactose
199 monohydrate can be prepared by allowing a supersaturated aqueous solution to crystallise
200 below 93.5°C (Gänzle, Haase, & Jelen, 2008).

201 Lactose is derived from whey, which is the liquid that remains after milk has been curdled
202 and strained, generally being a by-product in the process of cheese-making (Patel & Murthy,
203 2011b). The annual worldwide production of whey is estimated to exceed 80 million tons per
204 year, 40 - 50% of which is disposed of as sewage and the rest is used in human food and
205 animal feed production (Cheryan, 2005). The direct disposal of whey into the environment
206 can cause pollution, mainly due to its lactose content (approximately 5% w/w), which
207 contributes to over 80% of the biological oxygen demand (BOD) of whey (Patel & Murthy,
208 2009). Hence, the recovery of lactose from whey before discharging is necessary for the dairy
209 industry to prevent potential risks to human and animal health. The manufacture of lactose
210 also offers the advantage of improving the financial gain from whey utilisation by the
211 production of a valuable ingredient with diverse food and pharmaceutical applications (Patel
212 & Murthy, 2009), such as in bakery goods and confectionery products (contributing to
213 Maillard browning and enhancing flavour), meat products (filler and flavour enhancer), infant
214 formulas (increasing lactose content to match human milk) and pharmaceutical products
215 (filler and carrier).

216 The crystallisation of lactose is mainly carried out through evaporation to form
217 supersaturation, followed by agitation in cooling crystallisers (Nickerson, 1970). In the
218 industrial manufacture of lactose, whey usually undergoes purification steps prior to being
219 sent to the evaporators and crystallisers, since it contains impurities such as proteins, fats and
220 minerals. The presence of impurities usually interferes with molecular movement and
221 orientation, hence retarding or even inhibiting crystallisation. Proteins and salts contaminate
222 lactose and reduce its purity, and increase the viscosity of concentrated whey, making the

223 separation of crystallised lactose extremely difficult (Nickerson, 1970). Furthermore, the
224 presence of mineral impurities in the final lactose product renders it unsuitable for most food
225 and pharmaceutical applications, which require an ash content of <0.2 and <0.1%,
226 respectively (Lifran et al., 2011). Consequently, purification steps such as demineralisation,
227 heat coagulation, centrifugation, ultrafiltration and nanofiltration are initially performed to
228 bring whey to the highest possible level of lactose purity (Lifran et al., 2011; Patel & Murthy,
229 2012).

230 Two major difficulties are associated with the crystallisation of lactose: it is slow (Patel &
231 Murthy, 2010; Raghavan et al., 2001) and hardly controllable. The mixing generated by
232 agitators in cooling crystallisers is not uniform, which results in random fluctuations and
233 local zones of excessive supersaturation (Li, Wang, Bao, Guo, & Zhang, 2003). In these
234 regions, the nuclei and crystals cohere to each other and form agglomerates, which reduce
235 crystallinity, leading to an increase in the susceptibility of crystals to break and the
236 consequent formation of blunt edges. The agglomerates also trap traces of mother liquor
237 which detrimentally affects the purity of the final product (Li et al., 2006). The random
238 fluctuations in supersaturation also cause uneven growth in crystals (Dhumal et al., 2008),
239 leading to a wide CSD. Moreover, surface irregularities, such as crevices and dislocations,
240 may occur in crystals due to insufficient agitation, as some molecules may not have enough
241 time to find the correct orientation for binding (Dhumal et al., 2008; Li et al., 2006). Another
242 disadvantage of conventional crystallisation is the inefficient mixing which occurs mostly at
243 the interfaces of the macroscopic layers in the solution (Li et al., 2006). This results in
244 molecules in many regions of the solution having fewer opportunities to collide with each
245 other and form nuclei, which leads to a long induction time and poor nucleation rate.

246 A number of studies have been undertaken in the last few years to address the above-
247 mentioned issues and gain control over crystal properties and improve lactose recovery by
248 using ultrasound.

249 **5. The effect of ultrasound on nucleation rate of lactose**

250 The characterisation of the nucleation rate is of great importance in understanding and
251 consequently achieving better control over the crystallisation process of lactose. Many crystal

252 properties, such as crystal size and distribution as well as yield are highly influenced by the
253 nucleation rate (Jiang & ter Horst, 2010). The study of the nucleation rate of lactose is also
254 important since lactose crystallises slowly and any process that can accelerate nucleation will
255 result in huge cost savings for the lactose manufacturing industry by reducing the time
256 required to manufacture each batch of lactose. The cost saving would be further improved if
257 the nucleation of lactose can be induced at a low initial concentration, thus reducing
258 evaporation costs (Patel & Murthy, 2012).

259 Seeding and the use of anti-solvents have been used to try to accelerate the crystallisation of
260 lactose (Dhumal et al., 2008). Seeding accelerates crystallisation by inducing secondary
261 nucleation (forced crystallisation) and anti-solvents decrease solubility and hence increase
262 supersaturation, which is the driving force for crystallisation (Visser, 1982). Although these
263 methods improve the nucleation rate of lactose, both of them pose some major disadvantages.

264 The crystal habit is highly sensitive to the conditions and time of seed addition, which
265 restricts the applicability and reproducibility of seeding (Dhumal et al., 2008; Louhi-
266 Kultanen, Karjalainen, Rantanen, Huhtanen, & Kallas, 2006). The quality of the final product
267 is greatly influenced by the level of supersaturation present in the solution at the time of seed
268 addition. For instance, if the seeds are introduced too early, when the solution is still
269 undersaturated, a portion of the smaller seeds may dissolve. On the other hand, if seeding is
270 performed too late, it usually exhibits no effect on the crystal attributes (Louhi-Kultanen et
271 al., 2006). Another difficulty is that seeds are hard to disperse at the point of introduction into
272 the solution and tend to agglomerate (Guo et al., 2005).

273 The use of anti-solvents poses its own problems. It has been reported to result in (1) wide
274 batch-to-batch variability (O'Grady et al., 2007) because high supersaturation is formed
275 rapidly and uncontrollably (Luque de Castro & Priego-Capote, 2007), (2) formation of crystal
276 agglomerates (Guo et al., 2005) and (3) fine and irregularly shaped crystals (O'Grady et al.,
277 2007). This approach also requires expensive separation and purification steps to remove the
278 anti-solvents from the product (Genck, 2010).

279 The use of ultrasound in crystallisation has been argued to shorten the MSZW and reduce the
280 induction time. It can induce nucleation under low supersaturation conditions (inside the
281 MSZW) where spontaneous nucleation cannot otherwise occur, therefore removing the need

282 for seeding and reducing evaporation costs. It has been reported that under equal conditions,
283 the effect of ultrasound on inducing nucleation is higher than increasing concentration (Luque
284 de Castro & Priego-Capote, 2007; Patel & Murthy, 2009).

285 Ultrasound has been applied in lactose crystallisation primarily to accelerate nucleation.
286 However, most studies on the ultrasonic crystallisation of lactose have not reported its effects
287 on nucleation rate, presumably since it is very difficult to measure (Bund & Pandit, 2007b;
288 Dhumal et al., 2008; Kougoulos et al., 2010; Patel & Murthy, 2009; Patel & Murthy, 2010;
289 Patel & Murthy, 2011b) . Due to the scarcity of the techniques to monitor nucleation at its
290 molecular level, nucleation is often measured based on macroscopic properties (Hu, Hale,
291 Yang, & Wilson, 2001; Jiang & ter Horst, 2010; McLeod, 2007; Rodríguez-Hornedo &
292 Murphy, 1999).

293 Patel and Murthy (2011a) studied the effects of ultrasound on the anti-solvent crystallisation
294 of lactose using *n*-propanol. They estimated the total number of crystals per mL obtained at
295 the end of sonication time using the average roundness, density and mean diameter of
296 recovered lactose. They found that the number of crystals per mL increases and the size of
297 crystals decreases with an increase in sonication time (2 – 8 min), suggesting that continuous
298 sonication promotes nucleation rather than crystal growth. However, the authors did not
299 report the effect of the duration of sonication on nucleation rate, which is the rate of increase
300 in crystal number over time.

301 Zamanipoor et al. (2013) systematically studied the effects of ultrasonic variables including
302 amplitude and duration as well as lactose concentration on the nucleation rate of lactose in a
303 pure aqueous solution. Absorbance measurements were used as an indirect method for
304 estimating the nucleation rate. Nucleation rate was found to increase with an increase in
305 lactose concentration and sonication amplitude (Fig. 4). It was postulated that higher
306 supersaturation increases the driving force for crystallisation and higher amplitude promotes
307 cavitation effects leading to an increase in nucleation rate. The nucleation rate was found to
308 be insensitive to the duration of sonication. A 10.6-fold increase in nucleation rate in the
309 sonicated sample compared to the control was also observed, highlighting the effect of
310 ultrasound on the promotion of the nucleation rate.

311 **6. The effect of ultrasound on growth rate of lactose**

312 In addition to nucleation rate, growth rate is an important factor underlying the crystal
313 attributes, such as shape and size and recovery of lactose (Bund & Pandit, 2007c; Patil et al.,
314 2008). Hence, it is important to determine the effects of ultrasound and sonication conditions
315 on growth rate to increase control over the process of lactose crystallisation, thereby inducing
316 desirable crystal properties and yield. Most of the studies related to the ultrasonic
317 crystallisation of lactose have not reported the effect of ultrasound on growth rate (Bund &
318 Pandit, 2007b; Dhupal et al., 2008; Kougoulos et al., 2010; Patel & Murthy, 2009; Patel &
319 Murthy, 2010; Patel & Murthy, 2011b).

320 In a study of the effects of ultrasound on the anti-solvent crystallisation of lactose using *n*-
321 propanol, Patel and Murthy (2011a) varied the sonication time (2 – 8 min) and measured the
322 growth rate indirectly. They observed a reduction in crystal growth rate when the duration of
323 sonication was increased. They postulated that continuous sonication favours nucleation over
324 growth, resulting in final crystals of smaller size and a consequent reduction in growth rate.

325 A more comprehensive study by Zamanipour et al. (2013) reported the effects of ultrasonic
326 variables including amplitude and duration as well as lactose concentration on the growth rate
327 of lactose in a pure aqueous solution. Concentration was found to be the only factor that
328 significantly affected the crystal growth rate of lactose, and the growth rate increased with an
329 increase in concentration. An increase in concentration, at a certain temperature, increases the
330 supersaturation which is the driving force for crystallisation (Visser, 1982), promoting both
331 nucleation and growth rates.

332 **7. The effect of ultrasound on crystal size and CSD of lactose**

333 The crystal size and CSD of lactose are important properties from a manufacturing point of
334 view. It is critical to be able to tailor the desirable crystal size for different applications of
335 lactose with a minimal CSD. Obtaining a narrow CSD has been an important but difficult-to-
336 achieve target for lactose manufacturers since lactose crystallises uncontrollably. It is
337 desirable to minimise the CSD because achieving uniformity and reducing variability within
338 the manufactured product is advantageous from both processing and quality points of view.

339 Secondary nucleation occurs during the growth of lactose crystals, which results in the
340 formation of numerous small crystals and, consequently, in a wide CSD (Shi et al., 2006;
341 Wong et al., 2011b). These small crystals make the final processing (centrifugation, filtration,
342 washing, drying, etc.) difficult, resulting in reduced recovery and a final product of low
343 quality (Wong, et al., 2011a; Wong et al., 2011b). Consequently, the reduction of the number
344 of fine crystals and the narrowing of CSD will not only improve product quality but also will
345 promote lactose recovery. In addition, it is necessary for lactose manufacturers to control
346 crystal size and CSD to adhere to regulatory and marketing requirements. It is thus generally
347 desirable to operate the crystallisation process of lactose under conditions that promote
348 crystal growth and minimise secondary nucleation, leading to the formation of larger crystals
349 with narrow CSD (Shi et al., 2006; Wong et al., 2011b).

350 Crystallisation is the main method to produce pharmaceutical products (Patel & Murthy,
351 2011b). It is noteworthy to mention that the lactose crystals required for the manufacture of
352 pharmaceutical products should be of small size. It is more desirable to use fine lactose
353 crystals as fillers in tablets, since small crystal size allows better blending with other drug
354 ingredients (Dhumal et al., 2008). Lactose is the most commonly used carrier in dry powder
355 inhalers (DPIs) to deliver the drug to the lower airways in lungs (Kaialy, Ticehurst, &
356 Nokhodchi, 2012; Steckel, Markefka, teWierik, & Kammelar, 2004). The popularity of
357 lactose for DPI applications arises from a combination of appropriate characteristics: high
358 stability, safety, low cost and good flow properties (Kaialy et al., 2012; Smyth & Hickey,
359 2005). A reduction in lactose crystal size has been shown to improve the aerosolisation of
360 albuterol sulfate in Rotahaler® and budesonide in Spinhaler® (Steckel & Müller, 1997;
361 Zeng, Martin, Marriott, & Pritchard, 2001). Optimum drug delivery to the lung airways
362 occurs when particles are made in the size range of 2 – 6 µm (Pritchard, 2001). The shape of
363 particles also plays an important role because elongated crystals are dragged by the forces of
364 the air stream for longer periods of time (Dhumal et al., 2008) and more easily release the
365 drug particles during aerosolisation, due to potentially less drug-carrier interparticulate forces
366 (Kaialy & Nokhodchi, 2012; Nokhodchi, Kaialy, & Ticehurst, 2011). Zeng et al. (2001)
367 suggested that smaller crystal size, higher elongation ratio (needle-shape crystals) and
368 smoother surface contribute to better flow properties, dispersibility in air and the particles
369 remaining airborne, leading to deeper lung penetration and improved delivery of the drug.

370 Consequently, it is necessary to tailor small crystal size and achieve high elongation ratios
371 and surface smoothness if the lactose product is to be used in the pharmaceutical industry.

372 Micronisation has been used as a common, traditional method to reduce the crystal size of
373 lactose required for DPIs, which is performed by fluid air jet milling (Shariare, de Matas,
374 York, & Shao, 2011; Zeng et al., 2001). However, this technique is time-consuming, highly
375 energy-inefficient, and also results in the formation of highly charged particles with high
376 surface roughness, which significantly reduce the flowability required for efficient drug
377 delivery (Kougoulos et al., 2010; Leonelli & Mason, 2010; Zeng et al., 2001). Most
378 importantly, it increases the amorphous (glass) content of the carrier, leading to reduced flow
379 properties and dispersibility due to hygroscopicity (Ward & Schultz, 1995; Zeng et al., 2001).

380 Another approach which has been used for the manufacture of small crystals for DPIs is the
381 use of anti-solvents. It is well-established that the use of anti-solvents induces very rapid
382 formation of supersaturation, which accelerates the growth of crystal length and a reduction
383 in thickness, leading to the formation of smaller and elongated lactose crystals (Bund &
384 Pandit, 2007c; Patel & Murthy, 2009; Patel & Murthy, 2011b; Zeng et al., 2001), which are
385 suitable for pharmaceutical applications. While the use of anti-solvents favours the desirable
386 crystal size and shape for pharmaceutical applications, as described earlier, it also causes a
387 number of difficulties.

388 A number of authors have reported the effect of ultrasound on the size and CSD of lactose
389 crystals in solutions containing anti-solvents, with contrasting findings. It has been argued
390 that applying ultrasound causes better mixing and distribution of the anti-solvent throughout
391 the solution, leading to more uniform and rapid nucleation (Bund & Pandit, 2007c; Patel &
392 Murthy, 2009; Patel & Murthy, 2011b). Bund and Pandit (2007c) studied the crystal size and
393 CSD of lactose as affected by ultrasound in a solution containing ethanol as an anti-solvent,
394 and reported that protein is the dominant factor influencing CSD and crystal habit and that an
395 increase in protein content (0.2 – 0.8% w/v) widens the CSD. Furthermore, they found that an
396 increase in lactose concentration (11.5 – 17.5% w/v) reduces the crystal size and narrows the
397 CSD of lactose. These findings are consistent with those of Patel and Murthy (2009), who
398 reported a reduction in crystal size and CSD of lactose with an increase in lactose
399 concentration (12 – 16% w/v) in a sonicated solution containing acetone as an anti-solvent.

400 When crystallisation occurs in an anti-solvent system, an increase in lactose concentration
401 could work synergistically with the anti-solvent, resulting in a more rapid formation of
402 supersaturation and nucleation, leading to smaller crystal size. Interestingly, another study on
403 the effect of ultrasound on lactose crystallisation with ethanol as anti-solvent reported no
404 effect of lactose concentration (20 – 30% w/w) and sonication power (10 – 30 W) on crystal
405 size (Kougoulos et al., 2010). The contrasting findings that have been reported with anti-
406 solvent systems may be associated to the type of anti-solvent used and the experimental
407 conditions under which the studies were carried out.

408 Patel and Murthy (2011a) studied the effect of the duration of sonication on crystal size and
409 CSD of lactose in a solution containing *n*-propanol as anti-solvent, and observed a reduction
410 in crystal size and CSD when the sonication time increased from 2 to 8 min. Kougoulos et al.
411 (2010) also observed a decrease in the crystal size of lactose obtained from a reconstituted
412 lactose solution containing ethanol when the sonication duration increased from 10 to 120
413 min. The above authors argued that continuous sonication induces secondary nucleation by
414 cavitation disturbances at the crystal surfaces and breaks the already grown crystals, thus
415 reducing crystal size. A similar observation was made by Dhumal et al. (2008), who reported
416 obtaining smaller size crystals when an aqueous lactose solution was sonicated for 5 min
417 compared to when it was sonicated for 45 s, followed by 5 h of growth in a stagnant glycerin
418 solution (20% w/w). Consequently, it can be inferred that the continuous use of ultrasound
419 causes smaller crystals to be formed, regardless of the nature of the system investigated. This
420 suggests that it should be possible to tailor the desirable crystal size by adjusting the duration
421 of sonication (amongst other factors, such as lactose concentration).

422 **8. The effect of ultrasound on shape of lactose crystals**

423 Patel and Murthy (2011b) studied the ultrasonic crystallisation of lactose in the presence of
424 acetone as an anti-solvent and reported obtaining needle/rod shaped crystals by sonication vs.
425 tomahawk shape for the control (mechanically agitated at 1,000 rpm) and commercial lactose
426 samples. They associated this to the mixing effect of ultrasound, which allows uniform
427 supersaturation to be reached quickly, resulting in rapid crystallisation. Rapid crystallisation
428 accelerates the growth of the longest axis and a decrease in width, resulting in the formation

429 of needle-shaped, elongated crystals. Bund and Pandit (2007c) and Patel and Murthy (2011a)
430 also reported obtaining needle and/or rod crystal habits as affected by ultrasound in lactose
431 solutions containing the anti-solvents ethanol and *n*-propanol, respectively.

432 Kougoulos et al. (2010) studied ultrasonic crystallisation in a reconstituted lactose solution
433 using ethanol as an anti-solvent. The addition time of ethanol was found to be the most
434 significant factor on crystal habit and rod and needle-shaped crystals were mainly obtained
435 when ethanol was added to the solution rapidly (within 10 min from the start of sonication),
436 whereas a transition to tomahawk-shaped crystals was observed when the addition rate was
437 slowed down to 120 min, as shown in Fig. 5. Formation of the needle and rod crystal shapes
438 was associated to the rapid increase in supersaturation at the nucleation point in the presence
439 of ultrasound, which favours the formation of elongated crystals. It can be inferred from the
440 transition observed in Fig. 5 that reducing the effect of the anti-solvent (by adding it slowly)
441 leads to the formation of tomahawk-shaped crystals, which are not suitable for DPI
442 applications. However, these tomahawk-shaped crystals could be fit for other particular uses
443 such as food applications.

444 **9. The effect of ultrasound on lactose recovery**

445 Studying the ultrasonic crystallisation of lactose in aqueous solution, Zamanipoor et al., 2013
446 reported a significant, 5.6-fold increase in lactose recovery as a result of sonication. This was
447 consistent with Dhumal et al., 2008, who reported a significant positive effect of sonication
448 on the recovery of lactose in aqueous system. The main mechanism by which ultrasound
449 promotes the recovery of lactose is through its effects on mass transfer and nucleation rate, as
450 explained previously.

451 Bund and Pandit (2007c) and Patel and Murthy (2009) studied the ultrasonic crystallisation of
452 lactose in the presence of the anti-solvents ethanol and acetone, respectively. A strong
453 positive dependence of lactose recovery on the concentration of lactose (11.5 - 17.5% *w/v* and
454 12 - 16% *w/v*, respectively) was reported. This was explained by the fact that, in the presence
455 of cavitation, higher concentration causes higher supersaturation to be formed, resulting in a
456 higher nucleation rate and subsequent higher lactose recovery. By contrast, Patel and Murthy
457 (2010) studied the ultrasonic crystallisation of lactose from paneer whey after de-fatting and

458 de-proteination (reducing the protein content to less than 0.1% w/v) and reported no
459 significant effect of lactose concentration (5 – 15% w/w) on lactose recovery in a system
460 containing acetone as an anti-solvent. This could be attributed to the inhibitory effect of the
461 remaining protein and whey impurities on the nucleation rate, rendering the driving force of
462 higher supersaturation ineffective in inducing significant nucleation.

463 Bund and Pandit (2007c) and Patel and Murthy (2011a) also reported a significant negative
464 effect of the protein content of lactose solutions on lactose recovery. A reduction in yield was
465 observed with the increase in protein content (0 – 0.8% w/v and 0.2 – 0.8% w/v, respectively),
466 which was associated with a reduction in nucleation rate, since proteins are known to be
467 crystallisation inhibitors (Bund & Pandit, 2007c; Patel & Murthy, 2011a). However, even in
468 the presence of protein, the yield in the sonicated sample was higher than the control (Bund
469 & Pandit, 2007c).

470 Patel and Murthy (2009) reported that the recovery of lactose from reconstituted lactose
471 solutions improved with an increase in sonication time (2 – 8 min) in the presence of acetone.
472 A sharp increase in recovery was observed when the sonication time was increased from 2 to
473 4 min, but a subsequent increase from 4 to 8 min did not seem to have a large influence on
474 the yield. Other reports have also indicated a similar increase in yield with an increase in
475 sonication time, reaching a plateau with prolonged sonication (Bund & Pandit, 2007c; Patel
476 & Murthy, 2010; Patel & Murthy, 2011a). It was postulated that this could be due to a
477 decrease in supersaturation level as nucleation depletes the supersaturation over prolonged
478 sonication times (Patel & Murthy, 2011a). As the solution becomes depleted from
479 supersaturation, any longer sonication does not result in any further recovery.

480 **10. Industrial scale-up and future trends**

481 Research on the ultrasonic crystallisation of a number of chemical compounds has generally
482 shown promising results in terms of yield and product quality for the manufacturing industry.
483 However, the ultrasonic crystallisation of lactose is still in its infancy and further studies are
484 needed to fully characterise its parameters to maximise its benefits. In particular, future
485 research should aim to describe how lactose crystals respond to ultrasonic variables, as well
486 as to explore pathways to engineer and tailor desirable lactose crystals suitable for specific

487 applications, while maximising lactose recovery. Such laboratory-scale studies are essential
488 prior to investigating the scale-up of ultrasonic crystallisation of lactose. A critical factor,
489 among many others, is the duration of sonication required to achieve desirable results, since
490 the type of system required for scale-up (batch or continuous) will primarily depend on the
491 time period required for sonication.

492 There is a significant lack of knowledge in the area of ultrasonic crystallisation of lactose for
493 food applications, which generally require larger crystals with tomahawk shapes and narrow
494 CSD. Achieving the desirable lactose product quality and enhancing its recovery will not
495 only save the lactose manufacturing industry million dollars per year, but will also improve
496 the quality of the food products manufactured with lactose as an ingredient.

497 In addition to efforts aimed at implementing ultrasound in lactose crystallisation in the
498 industry, more powerful techniques to monitor nucleation and crystal growth need to be
499 developed to characterise how they respond to ultrasound. In addition, scale-up efforts will be
500 hampered if appropriate large-scale, food-grade devices able to deliver enough ultrasonic
501 power to lactose concentrates are not manufactured.

502 The penetration range of power ultrasound in a liquid is limited, and is estimated to be around
503 10 cm from the tip of a probe (Li et al., 2003; McCausland, Cains, & Martin, 2001).
504 Consequently, in order to sonicate the large volumes required for industrial manufacturing,
505 some technological developments have been initiated. These approaches include (1)
506 increasing the number of ultrasonic probes in a batch tank or pumping the liquid through a
507 number of probes in a tube (Li et al., 2003; Patel & Murthy, 2012), (2) positioning ‘tips’ or
508 small-area ultrasonic delivery devices in batch tanks or flow-cells and (3) arranging opposite
509 parallel transducers around a tube through which the liquid flows (Patel & Murthy, 2012)
510 (Fig. 6). The latter approach reduces the erosion of the equipment due to distributing the
511 power between a large number of transducers and concentrating the ultrasonic intensity
512 towards the centre of the cylinder rather than the vessel walls (Ashokkumar et al., 2010;
513 Rucroft et al., 2005). It also delivers ultrasonic energy as uniformly as possible throughout
514 the liquid volume, avoiding the phenomenon of cavitation blocking (acoustic decoupling),
515 which arises when high power is delivered to the liquid from a single delivery point (Rucroft
516 et al., 2005).

517 In addition to the physical aspects of sonication such as penetration range and the problem of
518 erosion, the potential chemical problem of formation of free radicals should be taken into
519 consideration. The local, high temperatures generated at the time of bubble collapse can lead
520 to the formation of highly reactive radicals, such as OH[•] and H[•] radicals in aqueous solution
521 (Ashokkumar et al., 2010). Depending on the type of food and processing conditions, these
522 free radicals may either enhance or reduce the quality of the sonicated food material.
523 However, the formation of free radicals can be minimised by sonicating at low frequencies
524 (20 KHz) or using appropriate radical scavengers at higher frequencies (Ashokkumar et al.,
525 2008).

526 The scale-up and adaptation of ultrasonic equipment for lactose manufacturing can be
527 challenging and requires careful consideration. Most importantly, the scale-up equipment
528 must be free from contamination and should not undergo any erosion induced by ultrasonic
529 cavitation. Eroded materials (e.g. titanium alloys) migrate to the crystal slurry and
530 contaminate it, rendering it inappropriate for food and pharmaceutical applications due to
531 their health hazards. There is consequently a need to investigate these contamination
532 processes and find ways to manufacture equipment that eliminates it. Furthermore, it is
533 necessary to study any detrimental chemical changes that ultrasound may induce in the
534 lactose crystal slurry and which could impact the sensory and/or hygienic properties of the
535 final lactose product.

536 **11. Conclusions**

537 Ultrasonic processing is an emerging technology which has been generally shown to improve
538 the crystallisation process of a number of chemical compounds, mainly improving control
539 over the crystal properties and recovery. However, the ultrasonic crystallisation of lactose has
540 not been researched extensively, especially in relation to food applications. Contrasting
541 findings have been reported for the use of ultrasound for different chemical compounds,
542 which necessitates in-depth investigation of its application for any crystallisation medium,
543 including lactose. Two major difficulties are associated with the crystallisation of lactose: it is
544 slow and hardly controllable, and different attempts have been focused to address these
545 issues. The use of ultrasound has been shown to improve the recovery of lactose, and

546 adjusting the processing variables such as duration of sonication and lactose concentration
547 can be useful in tailoring the desired lactose crystals for pharmaceutical applications, which
548 require small, elongated lactose crystals. By building upon knowledge gained from
549 pharmaceutical systems, it would be possible to engineer desirable lactose crystals for food
550 applications. The industrial scale-up of ultrasonic technology for the manufacture of lactose
551 still requires extensive laboratory-scale and pilot-scale studies directed at exploring pathways
552 to tailor desirable lactose crystals suitable for each application, while maximising lactose
553 recovery. In addition, appropriate large-scale, food- and pharmaceutical-grade equipment
554 able to deliver enough ultrasonic power to lactose concentrates need to be designed and
555 manufactured.

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755 **Figure Captions**

756 **Fig. 1.** Supersolubility diagram for lactose (reproduced from Wong et al., 2011b, with kind
757 permission from Elsevier; original data sources: Hunziker (1926) and Vu et al. (2003)).

758 **Fig. 2.** Classification of nucleation processes (reproduced from Luque de Castro & Priego-
759 Capote, 2007, with kind permission from Elsevier).

760 **Fig. 3.** Creation of (a) transient and (b) stable cavitation bubbles. C and R denote
761 compression and rarefaction, respectively (reproduced and modified from Santos et al., 2009,
762 with kind permissions from Wiley-VCH Verlag GmbH & Co. KGaA).

763 **Fig. 4.** Model for lactose nucleation rate as affected by ultrasound amplitude and
764 concentration (reproduced from Zamanipoor et al., 2013, with kind permission from Springer
765 Science and Business Media).

766 **Fig. 5.** Scanning electron microscopy (SEM) images showing the effect of the rate of ethanol
767 addition on lactose crystal habit in ultrasonic crystallisation: (a) addition within 10 min, (b)
768 addition within 60 min, and (c) addition within 120 min from the start of sonication. The
769 CSD in all cases seems large, presumably due to the addition of anti-solvent (reproduced
770 from Kougoulos et al., 2010, with kind permission from Elsevier).

771 **Fig. 6.** Prosonix Prosonitron® P750 large-scale ultrasonic flow cell composed of 42 bonded
772 transducers, capable of delivering 1,200 W of power at 20 kHz frequency. Printed with kind
773 permission from Prosonix Ltd., Oxford, UK.