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Effects of initial concentration and seeding procedure on crystallisation of orthorhombic paracetamol from ethanolic solution

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Abstract

The effects of some crystallisation conditions on the formation of orthorhombic paracetamol from ethanolic solution are examined when seeding technique is applied under fixed agitation (700 rpm) and harvesting time (30 min). Three equally spaced levels were used for initial concentration, C_I (30±4% w/v), for seeding temperature, T_S (7±7 °C) and for cooling temperature, T_C (−10±10 °C). The influences on parameters of temperature change in the crystallisation solution were quantitatively determined as were influences on crystal yield (*Y*%), crystal size and shape, and orthorhombic content of the final crystalline product. Conditions for improvement of *Y*% under reproducible formation of orthorhombic form were elucidated. It was found that *Y*% increases remarkably by increasing C_I but there is a corresponding decrease in the content of orthorhombic form. Increased content of monoclinic form is explained by nucleation under conditions of high C_I (34% w/v), low seeding temperature (below 7 °C) and long seeding time (more than 14.7 min) in addition to transformation of grown orthorhombic seeds. However, reproducible formation of pure orthorhombic form is possible at medium C_I (30% w/v) and optimal crystal yield (60.9% w/w) corresponds to −20 °C T_C and 0 °C seeding temperature. Crystal size is affected by all the crystallisation conditions due to the alteration in the degree of supersaturation, and consequently, in the nucleation and growth processes. Aspect ratio is affected due to the presence of less elongated monoclinic crystals. The fullness ratio increases with the cooling temperature but decreases with the initial concentration, probably because of secondary nucleation on the crystal surfaces or erosion due to initiation of transformation.
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Keywords: Crystallisation; Orthorhombic paracetamol; Box-Behnken design; Micromeritic properties; Supersaturation

1. Introduction

Preparation of orthorhombic paracetamol (form II) has attracted much interest because it may enable the tableting of pure paracetamol without incorporating binders. The orthorhombic paracetamol undergoes extensive fragmentation and therefore results in compacts of reduced elastic recovery after compression (Boldyrev et al., 1997; Joiris et al., 1998).

Recently orthorhombic paracetamol was obtained on a small scale by slow cooling of melts (Di Martino et al., 1997) and from ethanolic solution by applying seeding technique under controlled conditions (Nichols and Frampton, 1998). The first of these techniques is not suitable for large-scale production while the second was applied for larger scale (1-l crystalliser) and the effects of various crystallisation conditions were determined (Al-Zoubi et al., 2002a,b). It

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was found that orthorhombic paracetamol was reproducibly crystallised under certain conditions although it is "metastable" and converts to monoclinic (form I) upon extended contact with ethanol. Additionally, it was found that crystal yield (Y%) increased with agitation rate (AR) but decreased with cooling temperature T_C, while the content of orthorhombic form in the final product increased with AR and with T_C when AR was between 300 and 500 rpm. At higher agitation (700 rpm), the orthorhombic content was maximised and became independent of T_C (Al-Zoubi et al., 2002a). Under fixed agitation (700 rpm), the crystal yield increased with the harvesting time and the transformation of orthorhombic form to monoclinic was attributed to long contact with the solvent as well as to residual solvent evaporation during the drying process (Al-Zoubi et al., 2002b). Since crystal yield and solvent mediated transformation can be affected by the initial concentration of paracetamol in the ethanol solution and by the temperature at which seeds are added in combination with the cooling temperature applied, it was of interest to examine the effects of these crystallisation conditions.

In the present work, a Box-Behnken experimental design is applied. Three levels of initial concentration, C_I, of paracetamol are used (26, 30 and 34% w/v) under different cooling temperatures, T_C (−20, −10 and 0 °C) and the crystallisation liquid is seeded at different temperatures, T_S (0, 7 and 14 °C), because all are mainly related to the supersaturation rate. The crystalline product is totally harvested at a fixed time after seeding (30 min), its quality is evaluated and correlations are sought between the temperature change in the crystallisation liquid, the yield (Y%), the quality of the product (orthorhombic content and crystal size and shape) and the crystallisation conditions (C_I, T_S and T_C).

2. Materials and methods

The materials used in this study were as previously described (Al-Zoubi et al., 2002a,b) and the crystallisation procedure was modified in order to attain reproducible formation of orthorhombic form and crystal yield as high as possible.

For the preparation of orthorhombic seed crystals of paracetamol, a modified procedure of the method of Sohn (1990) was applied. About 5 mg of monoclinic paracetamol powder was completely melted on a glass microscope slide, at 190 °C, using a controlled temperature hot plate (Stuart Scientific, SH1D, UK). The slide was then rapidly cooled by placing it on a metallic block, at room temperature, and covered with a petri dish to avoid contamination. After complete crystallisation, an FT-IR identification test was performed on a small portion of the crystallised melt (2 mg), to ensure the existence of pure orthorhombic form (Al-Zoubi et al., 2002c). The remainder on the slide was kept in a desiccator over phosphorus pentoxide until required for seeding.

For the crystallisation from ethanolic solution, the procedure described previously (Al-Zoubi et al., 2002b) was modified regarding the initial amount of dissolved paracetamol and the seeding procedure. The apparatus consisted of a 1000-ml (7 cm × 30 cm) glass-vessel of jacketed wall connected with double valves to refrigerated and heated circulators, for flexible change and better control of the cooling. The temperature inside the solution was monitored by the use of a Pt 100 thermistor connected to an electronic polymeter (Handyscope, Tie Pie Engineering, The Netherlands) and to a computer equipped with suitable software. Solutions of different initial concentration (26, 30 and 34% w/v) were prepared by dissolving paracetamol (130, 150 and 170 g) in 500-ml ethanol kept under agitation, at a constant temperature of 50 °C, in an 1 l beaker, until a clear solution was obtained. The solutions were then carefully transferred, with a longneck glass funnel, into the crystallisation vessel, also thermostated at 50 °C by circulating warm water through the jacketed wall. Initially the solutions in the crystallising vessel were not agitated and their clarity was rechecked to ensure that no crystal formation occurred due to transfer. Then, circulation of the warm (50 °C) water was stopped, allowing the content of the jacketed wall and tubes to drain, and cooling was commenced by circulating cold fluid (mixture of water with anti-freeze solution) of certain temperature (−20, −10 and 0 °C).

The crystallisation solution was not seeded after cooling for certain time (as in previous work) but when it reached certain temperature and by employing confirmed orthorhombic paracetamol seeds. Seeding was performed by gently scraping the surface of the recrystallised paracetamol-melt (orthorhombic form)

that remained on the slide with a blade, adding about 0.5 mg of seed-crystals, and commencing agitation at fixed rate (700 rpm). This enabled independent alteration of seeding temperature and cooling temperature, since both these parameters can affect solvent mediated transformation. The range of seeding and cooling temperature selected in this study was based on the work already published for reproducible formation of orthorhombic form and relatively high crystal yield. Furthermore, the fixed agitation resulted in closer control of cooling rate than in the previous papers (Al-Zoubi et al., 2002a,b).

The crystals produced were harvested at certain time after seeding (30 min) by filtration under vacuum and dried as previously described (Al-Zoubi et al., 2002a). The drying equipment consisted of a 100 μm sieve placed under another sieve of 30 μm and on top of a metallic base having a side opening (air inlet). The harvested crystals were spread on the 100 μm sieve already placed on the base and the top sieve was fitted (allowing escape of air and preventing loss of crystals). Then air was blown, at room temperature, for 1 h; the 100 μm sieve with the crystals was subsequently removed and left in open-air for 24 h, to allow evaporation of any remaining traces of ethanol. Finally, the crystals were collected, weighed and kept in plastic bottles until required for examination.

Temperature of the crystallisation solution was measured and recorded throughout the cooling period with an accuracy of ±0.01 °C and parameters of its change were calculated. Data were collected at a rate of 30 points/min and graphs were plotted with and without paracetamol (reference). Fig. 1 represents typical plots. The temperature at which the angle between the tangents of the plots with and without paracetamol became greatest, T_m, was determined. Then the extrapolated point, T_d, was obtained as the intersection of the vertical passing from T_m and the reference plot (Nikolakakis et al., 2000). T_d is the ideal cooling temperature that corresponds to maximum rate of temperature deviation in the crystallisation solution and is considered an indication of maximum crystallisation rate. The time from circulation of cold fluid to seeding, t_S, and the time from seeding to maximum crystallisation rate, t_{max}, was determined and the apparent mean cooling rate, CR, was calculated from the time of seeding to the end of each crystallisation run as the difference between corresponding temperature divided by the duration.

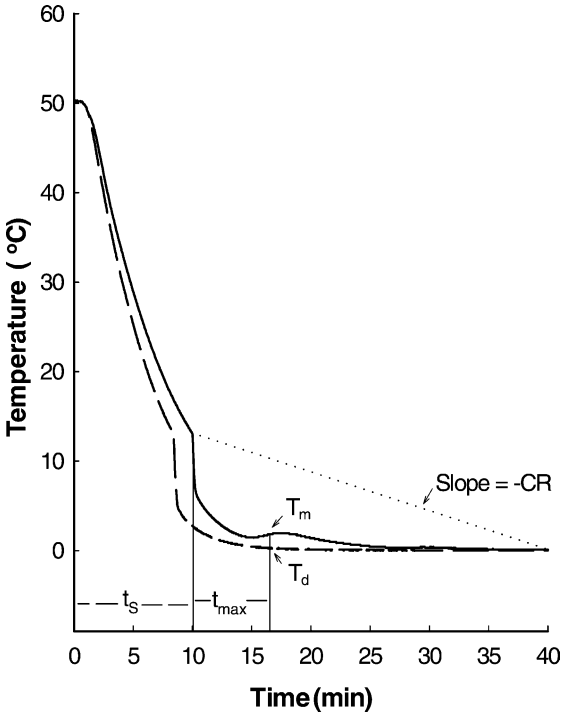

Fig. 1. Representative plots of temperature in the crystallisation solution with and without paracetamol (solid and dashed line) and parameters of its change (seeding time, t_S, time for maximum rate of temperature deviation, t_{max}, the corresponding ideal cooling temperature, T_d, and apparent mean cooling rate, CR). The experimental conditions were: C_I = 30% w/v, T_S = 14 °C and T_C = 0 °C.

Finally, the crystal yield (Y%) was calculated from the weight of crystals expressed as percentage of paracetamol dissolved initially, and the quality of the crystalline product was evaluated on the basis of the content of orthorhombic form and the size and shape of the crystals. For the quantitative analysis of the two forms (orthorhombic and monoclinic) a specially developed FT-IR method was employed (Al-Zoubi et al., 2002c). KBr disks (1% w/w) were prepared on a Specac hydraulic press and were analysed using a Perkin-Elmer FT-IR 1605 spectrophotometer. The intensity ratio of the 836 cm^{-1} band, which is attributed to the presence of both forms, to the 806 cm^{-1} monoclinic band was used and monoclinic molar fraction (X) was obtained from the equation:

$I_{836}/I_{806} = 0.515/X + 0.700$. Three determinations were made and the mean and standard deviation of the orthorhombic content were calculated. The mean diameters by number and the aspect and fullness ratio were determined as described by Nikolakakis et al. (2000). At least 500 crystals were measured in four optical fields of samples dispersed in paraffin oil by using an image processing and analysis system (Quantimet 500, Leica, Cambridge, UK).

2.1. Experimental design and statistical analysis

A Box-Behnken experimental design was followed in order to reduce the number of runs (12 plus replication of the central point, instead of 27 runs of a full factorial). Moreover, this design was selected because it is advantageous in cases where extreme points (at the vertices of the cubic region created by the upper and lower limits of each variable) are of little interest or impossible due to physical constrains. Furthermore, this design was selected because the main effects and the two-factor interactions are not aliases except in cases with high order interactions (Montgomery, 1996). Three factors or independent variables (initial concentration of paracetamol, C_I, seeding temperature, T_S, and cooling temperature, T_C), at three equally spaced levels (30 ± 4% w/v, 7 ± 7 °C and −10 ± 10 °C, respectively) were applied with two replications of the central point. The selection of the three levels was based on work already published (Al-Zoubi et al. 2002a,b) for the improvement of crystal yield by increasing the initial concentration and altering the seeding procedure.

Methodology of contour plots was applied in order to visualise the effects of the crystallisation factors and elucidate the conditions for improvement of crystal yield under reproducible formation of orthorhombic form. For ANOVA, the program SPSS 11.0 (Inc. Chicago, IL) was used. The experimental data were related to the variables by applying multiple linear regressions and fitting to second order polynomial equations. The equations were simplified by eliminating the statistically insignificant terms at the 95% level. The *t*-values and significance for the full models and coefficients of determination R^2 and significance of the simplified polynomials were obtained.

3. Results and discussion

Table 1 gives the results for the parameters of temperature change in the crystallisation solutions, together with crystal yield, which are mainly related to the supersaturation rate. Table 2 summarises the micromeritic properties (size and shape parameters) and the results of orthorhombic content for the

Table 1
Parameters of temperature change in the crystallisation solution determined under different crystallisation conditions

Initial concentration, C_I (%w/v)	Seeding temperature, T_S (°C)	Cooling temperature, T_C (°C)	Seeding time, t_S (min)	CR (°C/min)	T_d (°C)	t_{max} (min)	Crystal yield, *Y*%
26	7	−20	8.9	0.86	−17.36	10.2	48.2
26	0	−10	14.6	0.32	−9.15	14.1	42.4
26	14	−10	8.3	0.78	−8.85	11.7	43.9
26	7	0	14.7	0.23	0.32	12.1	32.6
30	0	−20	10.0	0.63	−18.65	6.0	60.9
30	14	−20	6.9	1.08	−14.15	5.6	58.1
30	7	−10	10.0	0.55	−9.18	6.4	54.8
30	7	−10	10.0	0.53	−9.07	6.1	53.6
30	7	−10	10.0	0.55	−9.17	6.2	54.5
30	1[a]	−1[a]	25.1	0.06	−0.88	8.1	49.7
30	14	0	10.0	0.46	0.54	6.4	49.1
34	7	−20	9.1	0.84	−14.70	5.8	63.2
34	0	−10	14.7	0.31	−7.26	5.4	58.4
34	14	−10	8.2	0.75	−4.84	4.8	58.7
34	7	0	15.1	0.22	1.75	5.0	53.0

[a] For the case where the cooling and seeding temperature were the same (0 °C), slight modification (±1 °C) was applied for the realisation of crystallisation.

Table 2
Content of orthorhombic form and size and shape of crystals obtained under different crystallisation conditions

Initial concentration, C_I (%w/v)	Seeding temperature, T_S (°C)	Cooling temperature, T_C (°C)	Orthorhombic content (%)	Crystal diameter (μm), mean ± S.D.	Aspect ratio	Fullness ratio
26	7	−20	100.0	89 ± 49	2.61	0.90
26	0	−10	100.0	97 ± 57	2.12	0.93
26	14	−10	100.0	108 ± 64	2.40	0.91
26	7	0	100.0	118 ± 70	1.85	0.95
30	0	−20	100.0	54 ± 28	2.69	0.88
30	14	−20	100.0	78 ± 43	2.49	0.89
30	7	−10	100.0	77 ± 33	2.39	0.91
30	7	−10	100.0	72 ± 36	2.52	0.90
30	7	−10	100.0	74 ± 32	2.51	0.89
30	1	−1	100.0	102 ± 57	2.24	0.91
30	14	0	100.0	86 ± 44	2.36	0.91
34	7	−20	89.0	61 ± 34	1.81	0.87
34	0	−10	51.1	69 ± 40	1.91	0.85
34	14	−10	96.2	77 ± 48	1.96	0.88
34	7	0	73.6	75 ± 46	1.84	0.88

S.D. for orthorhombic content was <5.3, for aspect ratio was 0.46–0.84 and for fullness ratio 0.04–0.06.

crystalline products, which are mainly affected by the nucleation and growth of crystals. The results of ANOVA (*t*-values and significance *P*), for the effects of the crystallisation conditions under examination on each parameter evaluated are shown in Table 3.

3.1. Temperature change in the crystallisation solution

From Table 1 it can be seen, as expected, that the time from the start of cooling until seeding, t_S, increases with increasing cooling temperature, T_C, and with decreasing seeding temperature, T_S. Table 1 also shows that the apparent mean cooling rate, CR, changes inversely with t_S. Furthermore, ANOVA (Table 3) shows that the effect of all the crystallisation conditions on t_S is significant and most significant is that of T_C followed by those of C_I and T_S. The polynomial equation that describes the seeding time, t_S, as a function of crystallisation conditions, after eliminating the insignificant terms at the 95% level becomes:

$$t_S = 72.479 - 3.652C_I + 0.458T_C - 0.749T_S - 1.944 \times 10^{-2}(T_S)(T_C) + 5.112 \times 10^{-3}(T_C)(C_I) + 6.203 \times 10^{-2}(C_I)^2 + 8.239 \times 10^{-3}(T_S)^2 + 9.028 \times 10^{-3}(T_C)^2, \quad (R^2 = 0.998,\ P < 0.001) \qquad (1)$$

Table 3
ANOVA (*t*-values and significance *P*) for parameters of temperature change in the crystallisation solution, crystal yield, content of orthorhombic form and size and shape of crystals obtained under different crystallisation conditions

Variable	*t*-value and significance *P* for:		
	Initial concentration, C_I (%w/v)	Seeding temperature, T_S (°C)	Cooling temperature, T_C (°C)
Seeding time, t_S	−8.436 (<0.001)	−8.141 (<0.001)	9.926 (<0.001)
T_d	−1.770 (0.137)	0.122 (0.908)	2.725 (0.042)
t_{max}	−4.925 (0.004)	−0.310 (0.769)	0.326 (0.757)
Crystal yield, *Y*%	4.624 (0.006)	−1.225 (0.275)	−2.394 (0.066)
Orthorhombic content	1.389 (0.224)	−1.366 (0.230)	−1.209 (0.281)
Diameter	−6.279 (0.002)	1.593 (0.172)	3.401 (0.029)
Aspect ratio	3.613 (0.015)	−1.017 (0.356)	−1.465 (0.203)
Fullness ratio	−0.243 (0.817)	−0.084 (0.937)	0.372 (0.725)

From Table 1 it can be seen that the ideal cooling temperature, T_d, which is considered as indication of maximum crystallisation rate (Nikolakakis et al., 2000), increases with the cooling temperature, the seeding temperature and the initial concentration of paracetamol as well. All these changes in T_d may be attributed to alteration of supersaturation. ANOVA (Table 3) shows that the significance of cooling effect on T_d is highest, followed by those of initial concentration and seeding temperature. Furthermore, the interaction term in the simplified polynomial (for $P > 0.051$, Eq. (2)) indicates that the effect of seeding temperature on T_d is influenced by the level of cooling temperature.

$$T_d = 48.424 - 3.611C_I + 0.757T_C + 5.836 \times 10^{-3}(T_C)(C_I) + 6.635 \times 10^{-2}(C_I)^2 - 1.325 \times 10^{-2}(T_S)(T_C), \quad (R^2 = 0.991, P < 0.001) \qquad (2)$$

The values of time since seeding until the maximum crystallisation rate (massive nucleation and crystal growth), t_{max} in Table 1, seem to decrease with increasing initial concentration, C_I, indicating quicker crystallisation at higher initial supersaturation. However, this decrease is higher when changing the levels of C_I from low (26% w/v) to medium (30% w/v) than that from medium to high (34% w/v). ANOVA (Table 3) shows that the significance of the initial concentration on t_{max} is remarkably higher than those of cooling and seeding temperature and the polynomial equation that shows the change of t_{max} with crystallisation conditions after correcting for the insignificant terms ($P > 0.05$) is:

$$t_{max} = 157.666 - 9.238C_I + 0.140(C_I)^2, \quad (R^2 = 0.901, P < 0.001) \qquad (3)$$

From all these, it is seen that, from the examined conditions, t_{max} is mainly controlled by the initial concentration.

3.2. Crystal yield

The values of crystal yield (Y%), in Table 1 and in Fig. 2a–c, presented as contour plots for medium crystallisation conditions (T_S, T_C and C_I, respectively), increase with increasing initial concentration (C_I) and decreasing cooling temperature (T_C). The increase of crystal yield from low to high level of C_I (14.8–20.4%) and from low to high level of T_C (10.0%, at highest T_S) corresponds to a remarkable increase in the weight of crystalline product, which justifies the applied design. The change of the crystal yield due to changing the seeding temperature (T_S), as seen from Fig. 2b and c and from Table 1, is relatively small and not specific. This is also seen from the relatively low significance level in Table 3 and from the absence of the terms of seeding temperature in the polynomial equation expressing the change of crystal yields with the crystallisation conditions after eliminating the insignificant terms at the 95% level (Eq. (4)).

$$Y\% = -252.789 + 18.015C_I - 0.583T_C - 0.266(C_I)^2, \quad (R^2 = 0.973, P < 0.001) \qquad (4)$$

The absence of significant effect on Y% by T_S means that it is not affecting significantly the supersaturation, although it might affect the growth rate of the seeds and the formation of nuclei (monoclinic or secondary orthorhombic). The high agitation applied may be suppressing the effect of T_S on both growth of seeds and secondary nucleation. Parallel change of T_S, initial concentration and agitation, at levels lower than 700 rpm, might be valuable in elucidating the conditions for growth of the orthorhombic seeds or for inhibition of monoclinic nucleation.

Regression analysis (full model) of the experimental data for Y% and parameters of temperature change in the crystallisation solution (t_{max}, CR and T_d) showed no significant effect of any of them ($R^2 = 0.950$, $P < 0.009$). The corresponding simplified polynomial equation, at the 95% significance level, is:

$$Y\% = 65.010 - 0.537T_d - 2.272t_{max}, \quad (R^2 = 0.900, P < 0.001) \qquad (5)$$

3.3. Content of orthorhombic form

As far as content of the orthorhombic form is concerned, it is seen from Table 2, that pure orthorhombic form was obtained for all the runs corresponding to the low and medium levels of initial concentration (C_I). At the high level of C_I, 34% w/v, mixtures of the two polymorphs result. This indicates that obtaining pure orthorhombic crystals in the applied range of T_S

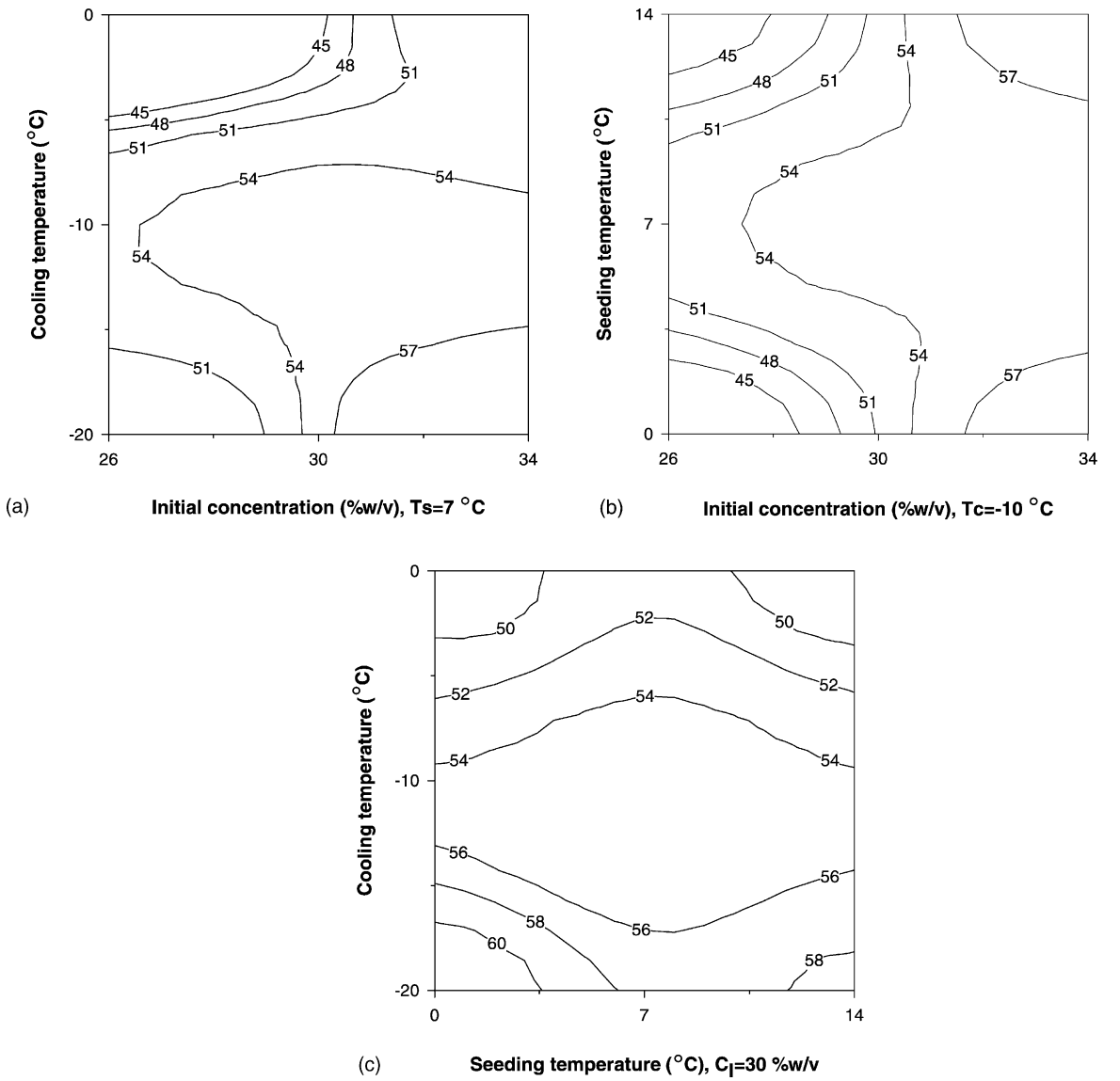

Fig. 2. (a–c) Contour plots showing the changes of crystal yield with the crystallisation conditions applied, at medium level of: seeding temperature (a), cooling temperature (b), and initial concentration (c).

(or corresponding seeding time) depends on the initial concentration, C_I. Also, from Table 2, it is seen that the orthorhombic content is decreased remarkably (from 96.2 to 51.1) by decreasing the seeding temperature, T_S (or by extending the seeding time, t_S), even at the medium level of T_C ($-10\,^{\circ}C$). This may be attributed to possible formation of monoclinic nuclei at the high level of initial concentration, C_I, although crystal formation was not visually noticed before the seeding. It is known that initial concentration or initial supersaturation decreases the critical nucleus size for primary nucleation and furthermore reduces the induction period (Mullin, 1993). Therefore, besides the transformation of orthorhombic crystals (grown from seeds), competition between the added orthorhombic seeds and monoclinic nuclei, possibly formed before or after the seeding, may exist at the high C_I. ANOVA (Table 3) shows balanced significance for the effects

of the crystallisation conditions examined. The polynomial equation expressing the change in orthorhombic content after correcting for the insignificant ($P > 0.051$) terms is:

$$\text{Orthorhombic content (\%)} = 127.646 - 1.763T_C - 7.057 \times 10^{-2}(C_I)^2 - 2.867T_S + 0.197(C_I)(T_S) + 0.158(T_C)(T_S), \quad (R^2 = 0.789,\ P = 0.007) \qquad (6)$$

The occurrence of changes in orthorhombic content at the high level of C_I only (Table 2), explains the relatively low coefficient of determination (R^2). Moreover, the absence of specific change in orthorhombic content with cooling temperature, T_C, or cooling rate (CR, given in Table 1), at the high level of C_I, may be due to the relative change in seeding temperature or seeding time. Furthermore, the balanced significance of the applied crystallisation conditions (Table 3) indicates

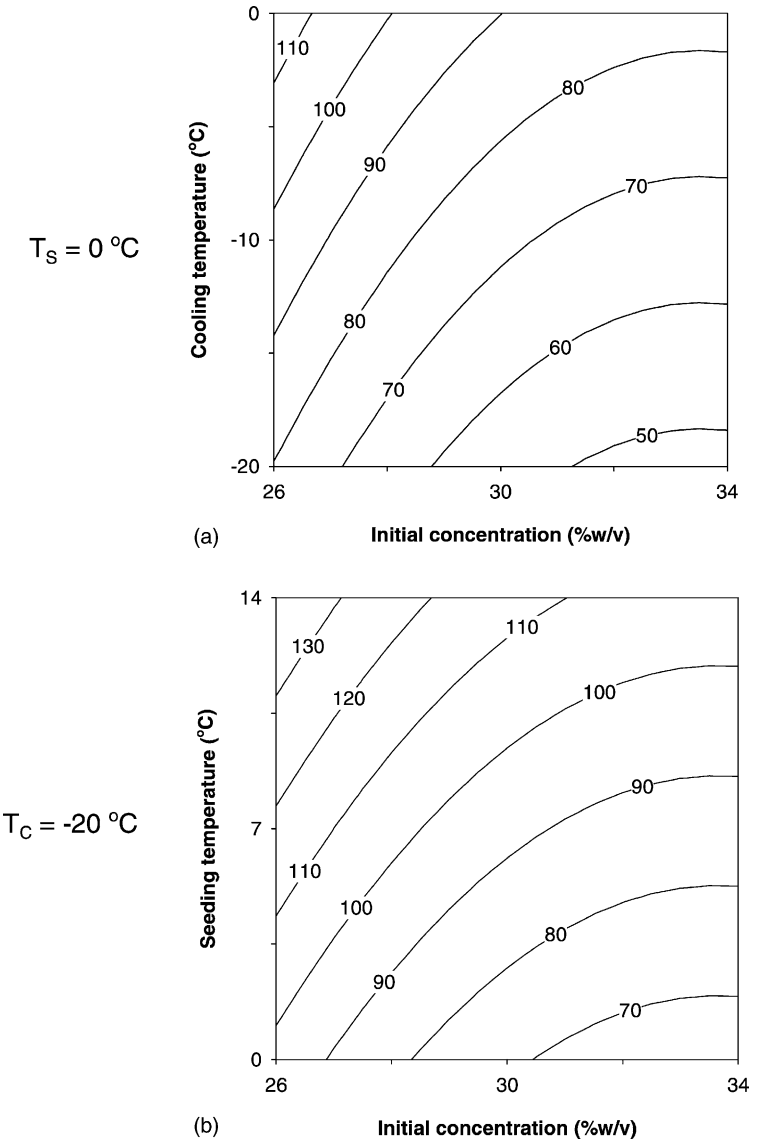

Fig. 3. (a–c) Representative contour plots showing the changes of mean diameter with the crystallisation conditions.

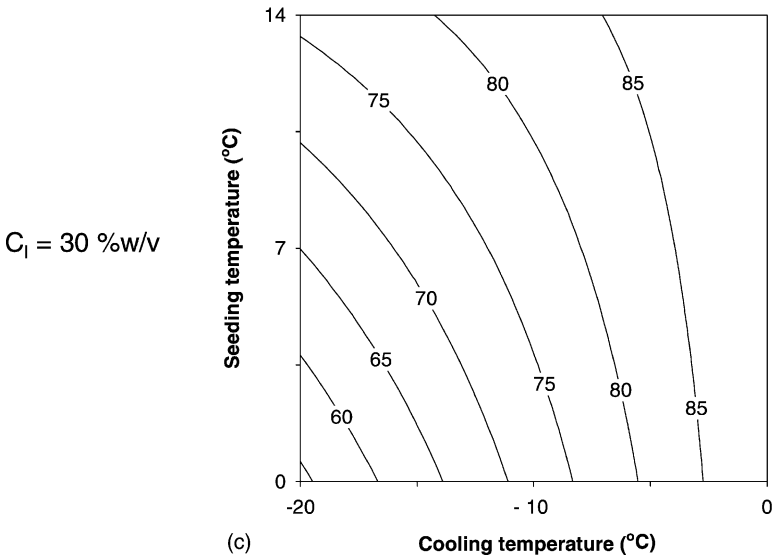

Fig. 3. (*Continued*).

that they all are important for undesired formation of monoclinic nuclei, for growth of orthorhombic seeds and for secondary nucleation, and finally, for the reproducible formation of orthorhombic paracetamol.

Decreasing T_C increases both the rate of cooling and supersaturation. This accelerates nucleation but reduces transformation (Mullin, 1993). Therefore, applying seeding at a certain time after cooling, and crystal harvesting at fixed time after seeding, it is expected that the decrease of T_C will either accelerate the undesired monoclinic nucleation and reduce the orthorhombic content or will reduce transformation and increase the orthorhombic content. The results in Table 2 indicate the second, since the orthorhombic content is comparatively higher (89.0%) for the lowest T_C (−20 °C) than that (73.6%) for the highest (0 °C), when medium temperature of seeding is applied (7 °C). On the contrary, at the longest seeding time (14.7 instead of 8.2 min), under fixed medium T_C (−10 °C), the orthorhombic content is lower (51.1% instead of 96.2%) indicating increased monoclinic nucleation either before or after seeding. In other words, all the above suggest that under high initial concentration (above 30%), low seeding temperature (below 7 °C) and long seeding time (more than 14.7 min) monoclinic nucleation occurs in addition to the growth of added orthorhombic seeds and the transformation to monoclinic.

From Tables 1 and 2 it is seen that the increase in crystal yield by increasing C_I is accompanied by decrease in the content of the orthorhombic form. However, reproducible formation of pure orthorhombic form at a relatively high crystal yield is possible for medium C_I (30% w/v) and optimal crystal yield (60.9% w/w) corresponds to −20 °C T_C and 0 °C seeding temperature (Fig. 2c).

Regression analysis (full model) of orthorhombic content and parameters of temperature change in the crystallisation solution (t_{max}, CR and T_d) showed that the significance of t_{max} is remarkably higher ($t = 2.693$, $P = 0.043$) than those of CR ($t = 1.856$, $P = 0.123$) and T_d ($t = 0.761$, $P = 0.481$). t_{max} is mainly controlled by the initial concentration (Eq. (3)) and the corresponding simplified polynomial equation, at the 90% significance level, is:

$$\text{Orthorhombic content (\%)} = -40.713 + 82.618\text{CR} + 25.721 t_{max} - 8.768(t_{max})(\text{CR}) - 1.072(t_{max})^2, \quad (R^2 = 0.552,\ P = 0.090) \qquad (7)$$

3.4. Size and shape of crystals

Regarding crystal size, it is seen from Table 2 and Fig. 3a–c that mean diameter increases with cooling

temperature, as expected, and decreases with initial concentration of paracetamol. As seen in Fig. 3a and b, this decrease is higher for the change in C_I from low (26% w/v) to medium (30% w/v) than from medium to high (34% w/v), possibly due to the predominance of nucleation over crystal growth at high degree of supersaturation (Granberg et al., 1999). ANOVA (Table 3) shows a significant effect on crystal diameter by both initial concentration and cooling temperature. Regarding the seeding temperature, only the term of interaction with cooling temperature was found significant at the 95% level (Eq. (8)). This is seen in Fig. 3c, in which increasing the cooling temperature reduces the effect of seeding temperature (and vice versa). A

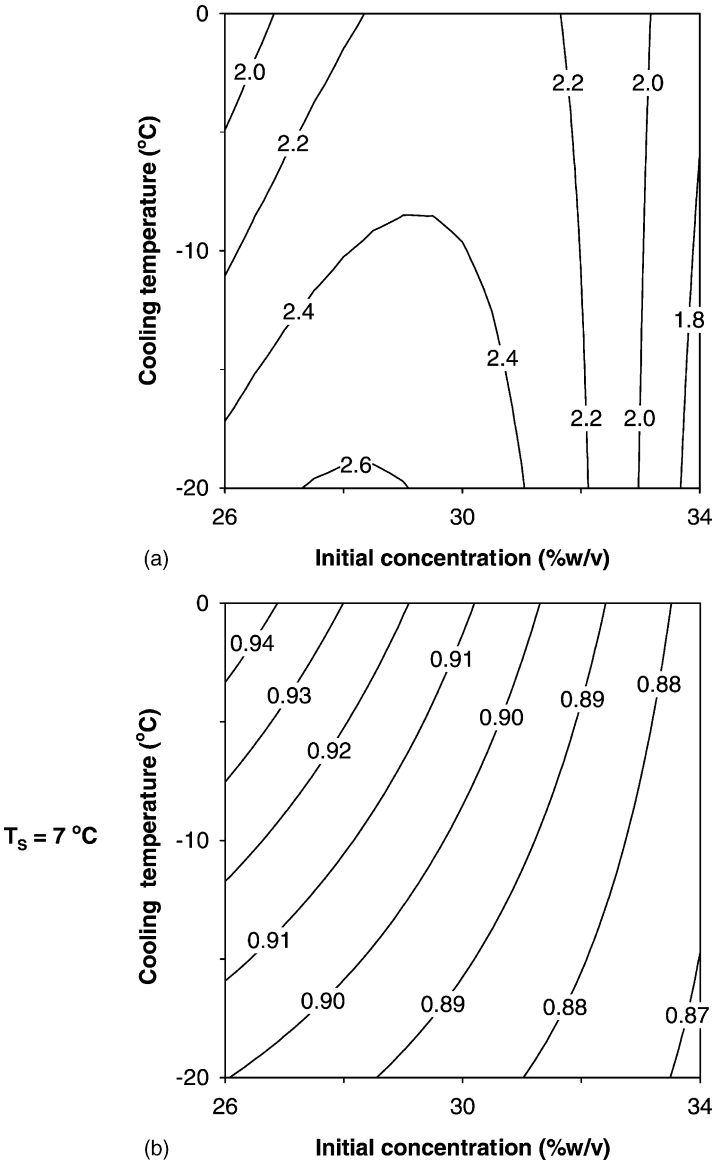

Fig. 4. (a and b) Contour plots for medium seeding temperature showing the changes with the crystallisation conditions of: (a) aspect ratio; and (b) fullness ratio.

similar significant interaction was noticed in the case of T_d (Eq. (2)), which indicates that the general effect of T_C and T_S on the size of crystal is related to their effect on T_d, because it represents the temperature where the maximum rate of crystallisation occurs.

The equation showing the relation of mean crystal diameter with the applied conditions after six removal steps (for $P > 0.051$) is:

$$\text{Diameter}\ (\mu\text{m}) = 873.264 - 48.491C_I + 0.740(C_I)^2 + 1.511T_C - 7.212\times10^{-2}(T_S)(T_C), \quad (R^2 = 0.974,\ P < 0.001) \qquad (8)$$

In previous work, it was found that the aspect ratio increases with the size of crystals and decreases

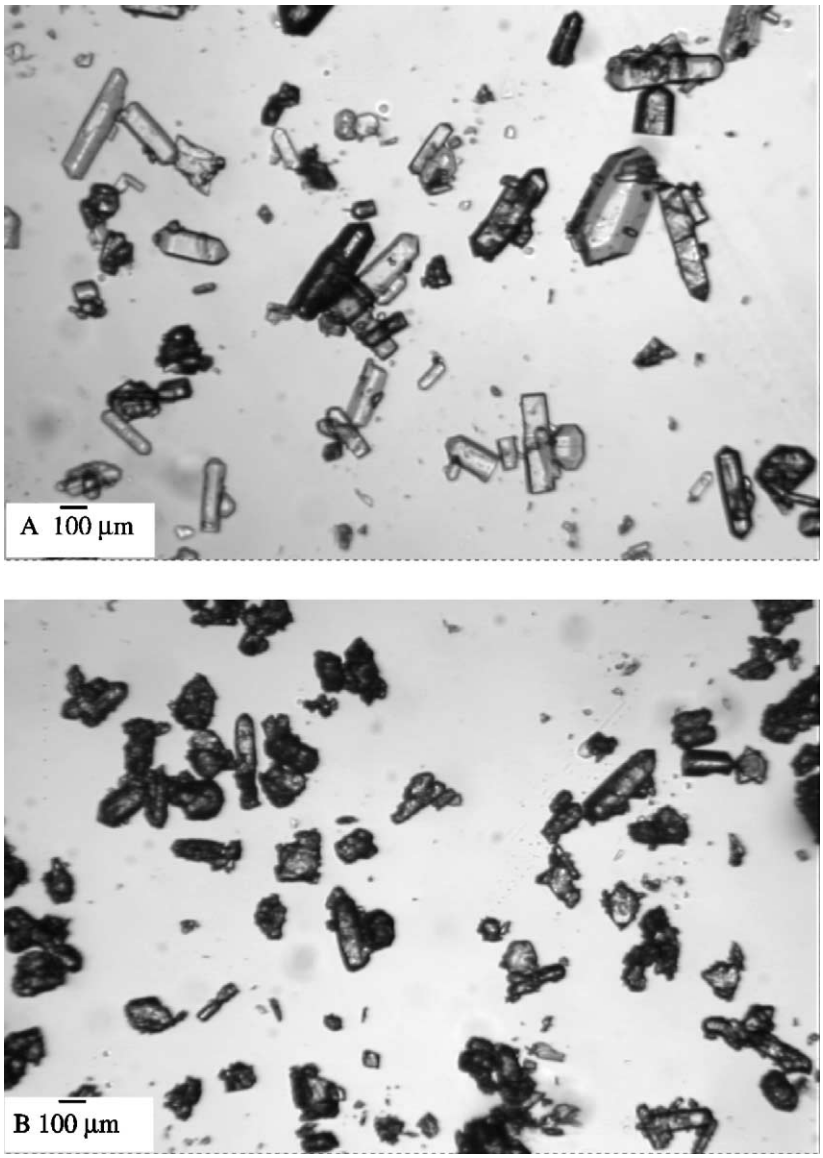

Fig. 5. Microphotographs of crystals prepared at fixed levels of T_S and T_C (14 and −10 °C, respectively) but different levels of C_I: (A) 26% w/v; and (B) 34% w/v.

with the cooling temperature, while the fullness ratio decreases with size and increases with cooling temperature. It was found also, that the effects of crystallisation conditions on crystal shape could be elucidated if size classification is taken into account, in order to eliminate the shape alterations due to crystal growth (Al-Zoubi et al., 2002b). Therefore, in this study crystals corresponding to a medium size range (75–150 μm), classified by image analysis (Quantimet 500, Leica, Cambridge, UK), were considered for evaluation of the effect of crystallisation conditions on the crystal shape and particularly of the initial concentration of paracetamol and of seeding temperature.

From Table 2 and Fig. 4a corresponding to medium T_S, it can be seen that the aspect ratio increases when C_I is increased from 26 to 30% w/v but decreases with the further increase in C_I to 34% w/v in addition to the decrease with the cooling temperature, which was expected. The increase from low to medium level of C_I, which corresponds to pure orthorhombic crystals, is attributed to increased supersaturation rate. The decrease in aspect ratio from medium to high level of C_I is probably due to formation of monoclinic crystals, which are less elongated than the orthorhombic, or to partial solving of the orthorhombic crystals. The most significant effect of crystallisation conditions on aspect ratio was that of initial concentration ($t = 3.613$, $P = 0.015$) followed by that of cooling and seeding temperature (Table 3). The simplified polynomial equation showing the change of aspect ratio with the crystallisation conditions (for $P > 0.051$) is:

$$\text{Aspect ratio} = -18.408 + 1.425C_I - 1.650 \times 10^{-2}T_C - 2.452 \times 10^{-2}(C_I)^2, \quad (R^2 = 0.807,\ P < 0.001) \qquad (9)$$

The fullness ratio, in Table 2 and Fig. 4b, increases with the cooling temperature and decreases with the initial concentration of paracetamol. A fullness ratio of less than 1 indicates more surface irregularity, with possible explanations of the observed reduction in fullness ratio being secondary nucleation on the crystal surfaces with increasing supersaturation and/or erosion of crystals due to initiation of transformation. None of the crystallisation conditions showed significant effect on the fullness ratio according to the ANOVA (Table 3) for the full polynomial model. The simplified equation showing the change of fullness ratio with the crystallisation conditions, at the 95% significance level, is:

$$\text{Fullness ratio} = 1.108 - 6.563 \times 10^{-3}C_I + 1.392 \times 10^{-3}T_C, \quad (R^2 = 0.827,\ P < 0.001) \qquad (10)$$

The changes in the crystal shape properties described above due to increase of C_I and the resulting transformation was confirmed in microphotographs of crystals obtained at fixed levels of T_S and T_C but different levels of C_I, shown in Fig. 5.

4. Conclusion

From the above results it can be concluded that for the crystallisation of orthorhombic paracetamol from ethanolic solution by seeding, the crystal yield increases remarkably by increasing the initial concentration but there is a parallel decrease in the content of orthorhombic form. Monoclinic nucleation occurs at high initial concentration (34% w/v), low seeding temperature (below 7 °C) and long seeding time (14.7 min) in addition to transformation of grown orthorhombic seeds. However, reproducible formation of pure orthorhombic form is possible at medium C_I (30% w/v), with an optimal crystal yield (60.9% w/w) corresponding to −20 °C T_C and 0 °C seeding temperature. Crystal size (mean diameter) is affected by all the crystallisation conditions due to the alteration in the degree of supersaturation, and consequently, in the nucleation and growth processes. Aspect ratio of the paracetamol crystals is affected due to the proportion of less elongated monoclinic crystals. Fullness ratio increases with the cooling temperature but decreases with the initial concentration, probably because of secondary nucleation on the crystal surfaces or erosion due to initiation of transformation.

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