ULTRASOUND ASSISTED COOLING CRYSTALLIZATION OF PARACETAMOL: EFFECT OF PROCESS PARAMETERS ON CRYSTAL YIELD, CRYSTAL SIZE AND OPTIMIZATION THROUGH BOX-BEHNKEN DESIGN

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ABSTRACT

The present study consists of ultrasound assisted cooling crystallization (USAC) of paracetamol. In this USAC study, the effect of various parameters such as pulsed ultrasound, amplitude, temperature, feed solution supersaturation, and crystallization time on the crystallized paracetamol yield and particle size was investigated. Further, the Box-Behnken experimental design (BBD) was utilized to conclude the most significant parameters affecting yield and crystal size. The optimum condition of BBD was found to be supersaturation level at 1.5, amplitude at 39.99 %, and sonication time at 19.47 min, and this condition, the yield of crystals was found to be 36.5 % and the crystal size of 10.793 µm as compared to raw paracetamol crystal size of 18.277 µm. Crystals were also determined by Differential Scanning Calorimetry (DSC), X-ray diffraction (XRD), and Scanning Electron Microscopy (SEM). Accurate prismatic shaped morphology of crystallized paracetamol crystals was observed in the USAC process. From the dissolution study, the drug release profile revealed that the crystallized paracetamol released 80.189 % in just 55 min whereas raw paracetamol crystals needed 90 min to release 81.243 % for the 150 mg tablet formulation.

Keywords: Ultrasound assisted crystallization (USAC), paracetamol, crystal size, Box-Behnken Design (BBD), dissolution.

INTRODUCTION

The pharmaceutical industry is becoming more and more interested in the significance of crystallization processes as it relates to the manufacture of medicinal components. The pharmaceutical drug has a significant role in the medical diagnosis, treatment, cure, and prevention of diseases [1]. These therapeutic medicines can be given orally, available in solid dosage forms as tablets and capsules in crystalline forms for drug delivery due to their high purity and patient acceptability. The bioavailability of these solid dosage forms is dependent on the physicochemical properties of active pharmaceutical ingredients i. e. API which is to be formulated [2]. To produce the desired results with adequate control of the necessary features, crystallization, the key unit of operation in the production of pharmaceutical solids, is generally utilized for the separation and purification process. In the crystallized form of the drug, the physical properties of the drug are determined along with its chemical and structural purity. Crystal engineering means its total analysis of pharmaceutical solids can give the huge potential for creating single crystals along with chemical, physical and mechanical properties. To control the size, shape, purity as well as yield of crystal products, the crystallization step is very important [3]. Over 90 % of active pharmaceutical ingredients (APIs) are produced using the crystallization methods of

purification. Most APIs with low water solubility have inherent drawbacks such as poor bioavailability and poor dissolution. The particle's surface area determines how quickly APIs dissolve, hence increasing the dissolution rate of oral drugs should be one of the goals of particle size reduction. Over the past decades, homogenization at high pressure and wet reduction processes have been mostly used but these are not suitable as mechanical friction produces high temperature and thereby causes the degradation of the products also it has a propensity to clump together when vacuum dried or lyophilized. Agglomeration is the reason for the deterioration of product quality, its compatibility, and stability and it leads to high chances of broadening the particle size distribution [4].

Earlier this decade, to overcome such problems, the crystallization process is assisted which mainly includes spray techniques or substrates that are not porous and have been utilized to formulate nano-sized APIs. The pharmaceutical industries place great importance on the mean size, size distribution, and shape of crystals since they directly affect how quickly drugs dissolve, their solubility, and tableting qualities, all of which have an impact on the drug's bioavailability. Additionally, the majority of active pharmaceutical ingredients (APIs) are polymorphic, which may have an additional impact on the API's filtration, tableting, dissolution, and bioavailability [5, 6].

The pharmaceutical sector has recently adopted USAC technology, which decreases the induction time of nuclei formation while increasing crystal yield and particle size. Benefits of sonocrystallization include enhanced morphology and polymorph selectivity, smaller Metastable Zone Width (MZW), more consistent and precise crystallization, minimum particle size distribution, and less induction times [7, 8]. The majority of the sonocrystallization studies documented in the literature are carried out using equipment that is readily accessible on the market and uses set ultrasonic frequencies between 20 and 100 kHz [9, 10]. It was discovered that ultrasonic promotes the precipitation of form by enhancing nucleation of the surface in addition to reducing the metastable zone width and time of induction [6]. The study of Jordens et al. investigated ultrasonic irradiation effects for frequencies between 41 to 1140 kHz [9]. In this study, the findings demonstrate that lower ultrasonic frequencies are preferred for accelerating nucleation and preventing deterioration. The largest reduction in this investigation was attained at 41 kHz, and a decline in the reduction was seen as the ultrasonic frequency was raised [9]. Gielen et al. demonstrated that cooling crystallization under sonication lowered the size of paracetamol to 50 μm and distribution was 70 - 140 μm after sonication nucleation was detected [11]. Vancleef et al. also studied the introduction of an ultrasound probe which leads to the form paracetamol having a particle size of 50 - 75 μm [12].

Paracetamol i.e. acetaminophen is the most used antipyretic drug and analgesic drug which is available in both solid as well as liquid dosage forms. Solid dosage forms include tablets capsules and suppositories and liquid dosage forms are solutions and suspensions. There exist 3 crystal polymorphic structures for paracetamol it includes monoclinic (form I), orthorhombic (form II), and an unstable phase (form III) these have different stability conditions [13 - 16]. Form I has inadequate technology and biological characteristics, such as flowing ability, compactibility, wettability, and dissolving rate, even though it is stable. Form II is suited for direct compression to form tablets without binders and may undergo plastic deformation [17]. Nichols and Frampton focused on creating form II from ethanol solutions in small-scale batch crystallizers with the help of seed crystals made by crystallizing from the melt, but it has drawbacks when used on a big basis. Hence To halt the transition process, they advised crystallization at temperatures below 5°C [16]. Dissolution rates of paracetamol can be increased by varying the size of particles of the drug which is applied for injectable dosage forms. USAC is the method by which particle size can be managed. Hence, in this study, paracetamol is chosen as a model drug.

In the past, paracetamol was crystallized using ultrasound to demonstrate how different ultrasonic frequencies affected the MZW and how paracetamol degraded. The MZW decreases with ultrasonic frequency, and higher frequencies considerably accelerate the degradation of paracetamol, according to the results [6, 9]. It has also been observed that sonication, a carrier-free method for improving the solubility of pharmaceuticals that aren't very soluble, can cause paracetamol to melt and crystallize [18]. Another recent study demonstrates that a monoclinic polymorph (form I) of paracetamol with a smaller crystal size is created by antisolvent

sonocrystallization of paracetamol, which led to noticeably better compaction behaviour [19]. While it has been reported that orthorhombic (form II) crystals were also observed and Bhangu et al. studied USAC of paracetamol where crystal size declined from 170 to 13 μm, induction time was lowered from 360 to 30 s, and size distribution was observed as narrow [20]. Only with the ultrasound crystallization form I as well as form II of paracetamol was obtained [6]. Mori et al. also studied ultrasonic irradiation for acetaminophen, where they found that without ultrasonication crystallization did not occur but when ultrasonication at 28 kHz was utilized; form II of acetaminophen was crystallized. Hence, it was concluded that one efficient method for selectively crystallizing the metastable phase is ultrasonic irradiation [21].

In this USAC study, the effect of various parameters on crystal size and yield of crystalized paracetamol active pharmaceutical ingredient (API) was done by parametric study. Moreover, to identify the most important factors and optimum conditions, the Box-Behnken experimental design (BBD) was applied.

EXPERIMENTAL

Materials

Paracetamol API was purchased from Panchsheel Pharmaceutical Pvt. Ltd. (Ahmedabad, Gujarat). The sodium chloride (NaCl), sodium bicarbonate, citric acid monohydrate, stearic acid, and lactose monohydrate were purchased from Loba Chemie Pvt. Ltd. (Mumbai, Maharashtra). All analytical-grade chemicals were used without further purification.

Methods

A schematic representation of the experimental set-up for Ultrasound assisted crystallization (USAC) of paracetamol is represented in Fig. 1. For the experiments of USAC, paracetamol solution was introduced in the feed solution tank or crystallizer Vibra Cell, Model VCX 700, Connecticut, USA). Approximately, a 13.0 mm diameter ultrasonic probe was immersed in the 8.0 mm depth of a solution.

A sample of paracetamol solution with varying supersaturations (1.3, 1.4, and 1.5) was made by mixing it in 150 mL of water at 65°C and swirling at a speed of 500 ± 10 rpm. Filter the solution to remove contaminants and any undissolved paracetamol after cooling it to room temperature. The solution was then added to a jacketed crystallizer with a heating and cooling system to maintain a temperature of 20°C being stirred at a speed of 200 ±10 rpm by magnetic stirring equipment (REMI, 2MLH). For all experiments, the ultrasound amplitude of 20 - 40 % and the pulsed ultrasound of 15 - 50 % were set. The run time of the experiment was 60 min. Crystals were formed and collected for drying at 60°C for 12 h. The percentage yield of crystallization was obtained at different parametric conditions. Various parameters used

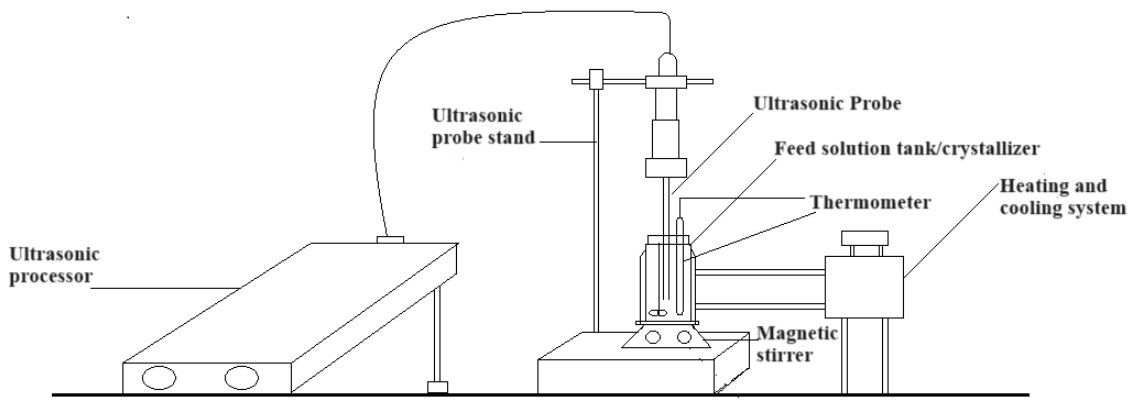

Fig. 1. Schematic representation of experimental setup for USAC of paracetamol.

Table 1. Various parameters for the experimental study of USAC paracetamol.

Sr. No.	Parameter	Unit	Range
1	Amplitude	%	20 - 40
2	Pulsed ultrasound	%	15 - 50
3	Supersaturation	-	1.3 - 1.5
4	Experiment run time	h	1 - 3
5	Feed solution temperature	°C	20 - 30

in the present study have been represented in Table 1.

Test methods

Particle Size Distribution

The particle size distribution and mean crystal size of paracetamol were determined with the use of Dynamic Light Scattering (DLS); Mastersizer 2000 Ver.5.61, Malvern Instruments Ltd, UK).

Differential Scanning Calorimetry (DSC)

The DSC was applied for analysing the thermal behaviour of raw and crystallized paracetamol. At about 30 - 300°C samples were heated at a rate of 10°C min^{-1} with a dry nitrogen flow rate of 25 mL min^{-1}. Standard sample pans made of aluminium were employed. For analysis, a hermetically sealed sample of approximately 1.8 - 2.0 mg was used. Using an indium standard, the DSC temperature and enthalpic scale were calibrated.

X-ray Diffraction (XRD) analysis

Using an XRD-diffractometer (Rigaku D/max 2200, Japan) having Cu kα radiation (λ = 1.54060 Å) filtered at an applied voltage of 40 kV with a current of 40.0 mA, an examination of X-ray diffraction was conducted. By application of a sample holder, the analysis was performed with angular scan mode was run with a step size of 2° and a pace of two seconds per step on 2Θ from 10° - 90°.

Scanning Electron Microscopy (SEM)

Model of HITACHI, S-3400N was utilized to create electron micrographs of paracetamol crystals. An electron gun was used where electrons from it were accelerated onto the specimen at a voltage of 1 - 15 kV. Before observation, on a piece of metal stub sticky tape, the samples were adhered to and coated with gold using a sputter coater in an argon atmosphere.

Table 2. Ingredients for paracetamol tablet formulation.

Ingredients	Formulations, mg	
	Raw paracetamol	USAC paracetamol
Paracetamol	150	150
Sodium bicarbonate	60	60
Citric acid monohydrate	7.5	7.5
Lactose monohydrate	15	15
Stearic acid	2.4	2.4

Tablet formulation

A tablet of raw paracetamol and USAC paracetamol was formulated by using a manual hydraulic laboratory press. The formulation of a tablet with an ingredient list is shown in Table 2. The sodium bicarbonate and citric acid monohydrate were used as pH-modulating agents, stearic acid was used as a solubilizing agent, and lactose monohydrate was used as a disintegrant for dissolution rate enhancement purposes with paracetamol API. Each dosage formulation i.e., 150 mg was compacted to form each tablet of 13 mm die. The load for the tablet was set at 150 kg cm^{-2} for 2 - 4 min.

Experimental design for USAC paracetamol

Box-Behnken design of three-factor and three-level was applied for the evaluation of independent variables on the percentage yield of crystallization. Each level of selection was based on the experimental trials where investigations were carried out to ascertain how process variables affect paracetamol crystallization. The Supersaturation (A), Amplitude (B), and Sonication time (C) were chosen as the independent variables and varied at 3 levels (-1, 0, and 1), respectively. The percentage yield (Y1), and particle size (Y2) of paracetamol were dependent variables. Analysis of statistical data from an experiment was examined using design-expert Software (Version 10.1). The framework of design is shown in Table 3.

Dissolution study of USAC paracetamol

About 900 mL medium-filled dissolving vessel was used for the dissolution investigation. The medium used for dissolution consists of 0.1 N HCl and has a pH of 1.2.

Table 3. Variables in a Box-Behnken design.

Independent variables	Coded symbol	Levels used		
		(-1)	(0)	(1)
Supersaturation	A	1.3	1.4	1.5
Amplitude, %	B	20	30	40
Sonication time, min	C	10	20	30
Dependent variables				
Yield, %	Y1			
Particle size, µm	Y2			

Stirring paddle with a 50 ± 0.5 rpm speed and a 37.5°C dissolving medium temperature. At the predetermined intervals (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, and 120 min), a 5 mL sample of the aliquot was taken from each vessel and the same amount was replenished by fresh medium for maintenance of sink condition. After that, a captive filter of a 0.2 µm pore size was used to filter the samples. All samples were examined to determine the drug content using a Thermo Scientific Evolution 201 UV-Visible Spectrophotometer at a wavelength of 243 nm after filtering and diluting them. It was calibrated to the optimal wavelength. Each experiment was performed twice to carried out to reduce the standard error. The percentage of the drug released at regular intervals was calculated and compared to the absorbance of the reference paracetamol solution treated under the same conditions. By use of 0.1 N HCl and standard drug solutions, the calibration curve was made. All formulations under study underwent the homogeneity of the content test at dosages of 150 mg and 100 mg. For the standard calibration curve, paracetamol 10 mg accurately weighed was diluted in 100 mL of 0.1 N HCl to create a 100 parts per million (ppm) stock solution. All these solutions' The absorbance of all these solutions was measured at 243 nm using a UV-Vis Spectrophotometer utilizing aliquots of 2, 4, 6, 8, 10, 12, and 13 ppm.

RESULTS AND DISCUSSION

Effect of amplitude

The feed solution supersaturation was kept at 1.4, the pulsed ultrasound intensity at 35 %, the feed solution temperature at 20°C, and the experimental run time of 1 h. The amplitude varied from 20 - 40 %. Fig. 2 represents the impact of amplitude on the percentage yield and particle size of paracetamol crystals for USAC.

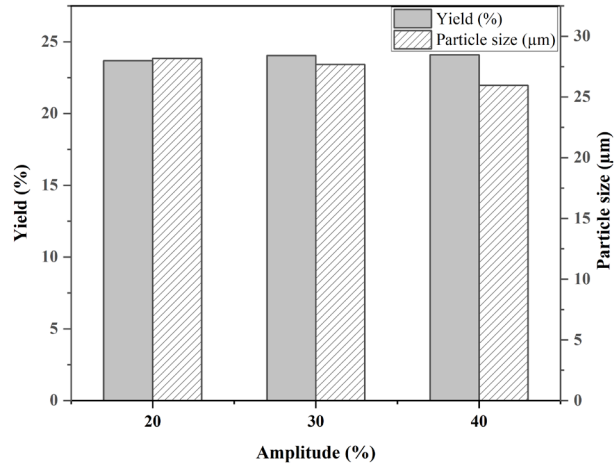

Fig. 2. Effect of amplitude on paracetamol crystal yield and particle size.

It was observed that the percentage yield increased from 23.69 % to 24.09 % with an increase in the amplitude from 20 % to 40 % respectively. Application of ultrasound at the time of the cooling crystallization of paracetamol at a particular supersaturation level generates the cavitation phenomena which further enhances the supersaturation level. In paracetamol solution, nucleation has reportedly been observed before 30°C. The ultrasound processor's vibrational amplitude and sonication time could be the operating parameters for ultrasonic irradiation [4]. The nature of the mass transfer is a key feature controlling the nucleation and growth rate and is influenced by the amplitude intensity. Acoustic cavitation and micro-turbulence are two factors that affect mass transport in solutions and have various effects on the process of crystallization. The nucleation rate is anticipated to rise because of the bubbles produced by sonic cavitation acting as heterogeneous nucleation

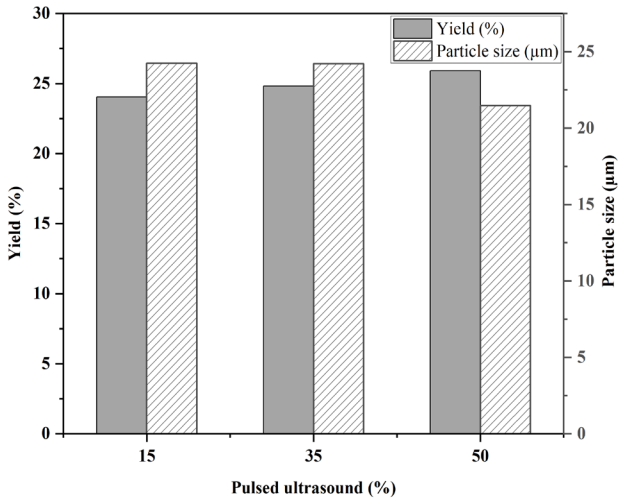

Fig. 3. Effect of pulsed ultrasound on paracetamol crystal yield and particle size.

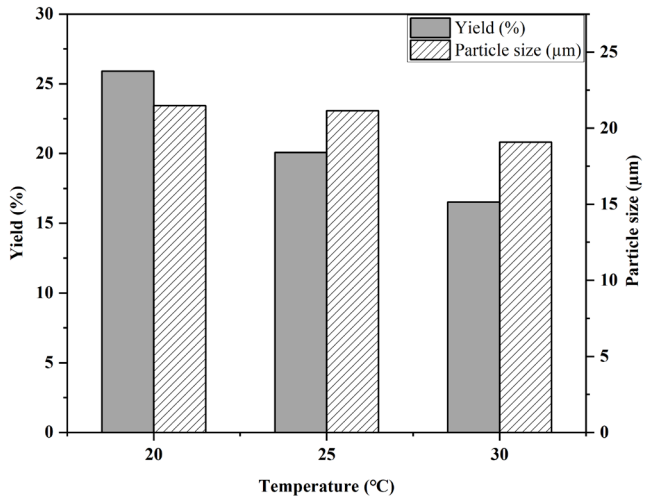

Fig. 4. Effect of USAC temperature on paracetamol crystal yield and particle size.

sites. Additionally, micro-turbulence speeds up the crystal growth rate by reducing the diffusion boundary layer distance between the bulk solution and the crystal growth surface [4, 22]. Energy input at higher amplitudes enhances the level of supersaturation which in turn enhances the nucleation rate and causes a rise in the percentage yield of paracetamol crystals.

In the case of particle size, as an ultrasonic amplitude of varying intensity is applied in cooling crystallization, the particle size of the crystal is reduced by increasing the ultrasonic amplitude. Due to higher amplitude, cavitation intensity is high which leads to more nuclei formation in the crystallization process and turbulence generated to a greater extent, seizures growth of crystal and leads to breakage particles, resulting in smaller particle size obtained [23, 24]. Experimental result shows that increase in the amplitude from 20 % to 40 %, particle size can be reduced from 28.18 μm to 25.95 μm respectively.

Effect of Pulsed Ultrasound

The impact of pulsed ultrasound on the percentage yield and particle size of paracetamol crystals under USAC is shown in Fig. 3. The pulsed ultrasound was varied from 15 - 50 %. An oscillating bubble population and a dissolving bubble population are both present in the sonicator vessel in a pulse-minimum environment. Compared to continuous sonication, the presence of a pulse threshold significantly reduces the amount of ultrasonic energy used [11]. It can be observed that with the increase in the pulsed ultrasound from 15 % to 50 %, the percentage yield of paracetamol crystals was increased from 24.69 % to 25.91 %, and particle size reduced from 24.25 μm to 21.49 μm respectively.

Effect of temperature

The feed solution supersaturation was 1.4, the pulsed ultrasound was kept at 35 %, and the amplitude was kept at 30 % to study the effect of temperature on the yield of paracetamol crystals under USAC. The temperature varied from 20 - 30°C.

The effect of temperature on the yield of paracetamol crystals and particle size is depicted in Fig. 4. It was found that the yield of paracetamol crystals under USAC and particle size reduced with a rise in the temperature. The maximum yield of paracetamol crystals was found to be 25.91 % at 20°C in the presence of ultrasound while at the higher temperature, a 16.33 % yield was observed. The particle size decreased from 21.48 μm to 19.08 μm for the USAC temperature at 20°C to 30°C respectively. When the ultrasound energy was supplied at the constant condition, the higher level of supersaturation generated at lower temperatures was due to a reduction in the solubility of paracetamol in the solvent and thereby maximum yield with larger particle size observed at low temperatures [4, 25].

Effect of feed solution supersaturation

For the study of supersaturation levels on the yield of paracetamol crystals and particle size under the USAC

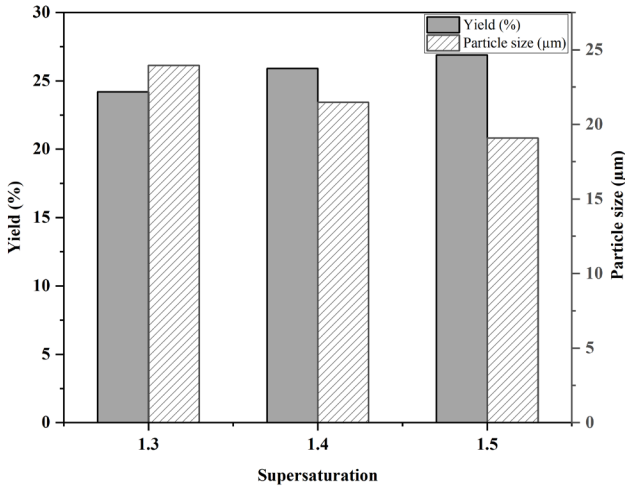

Fig. 5. Effect of feed solution supersaturation on paracetamol crystal yield and particle size.

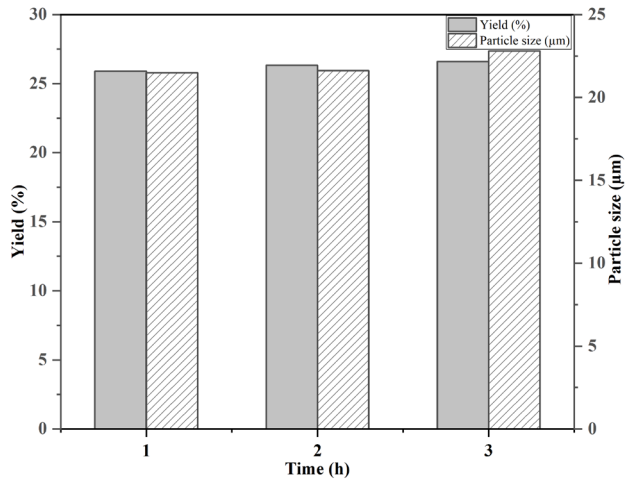

Fig. 6. Effect of time on paracetamol crystal yield and particle size.

as shown in Fig. 5, the supersaturation levels were varied from 1.3 - 1.5 by keeping the amplitude at 30 %, and pulsed ultrasound at 35 %.

It was detected that the yield of paracetamol crystals was increased from 24.20 % to 26.90 % with an increase in the supersaturation level from 1.3 to 1.5 and particle size reduced from 23.96 µm to 19.08 µm respectively. With the help of ultrasound, an increase in the supersaturation level raises the nucleation rate rapidly, and thereby a large number of nuclei is produced. Thus it leads to a high yield of paracetamol with smaller particle size due to limiting crystal growth at a high supersaturation level [4, 26].

Effect of time

To study the effect of crystallization time on the yield of paracetamol crystals and particle size as depicted in Fig. 6, it varied from 1 - 3 h by keeping the feed solution supersaturation at 1.4, the pulsed ultrasound at 35 %, and amplitude at 30 %.

The yield of paracetamol crystals and particle size increased slightly. The yield of paracetamol crystals was found to be 25.91 % and 26.61 % at 1 h and 3 h of crystallization time and particle size increased from 21.49 to 22.81 µm respectively. It indicated that the required supersaturation level to enhance the yield of paracetamol crystals might be achieved in the initial 1 h crystallization time and then there was a gradual increase in the yield and particle size with an increase in the crystallization time in the presence of ultrasound. In the literature, it was reported that sonication was undesirable when induction time was very short and without sonication, nucleation can take place before the desired supersaturation is reached. In the experiment, it was revealed that most induction time was less than 1 h [4].

Box-Behnken Design of Experiment for USAC

It was observed that the required supersaturation level to enhance the yield of paracetamol crystals might be achieved in the initial 1 h crystallization time and therefore, for the further study of the effect of parameters on the yield and crystal size of paracetamol, the time of crystallization was kept under 1 h. The Box-Behnken experimental design was utilized to carry out this study. In this BBD design, experiments were carried out at three factors at their three level. i.e. supersaturation (factor A) at 1.3, 1.4, and 1.5, amplitude (factor B) at 20, 30, and 40, and sonication time (factor C) at 20, 30, and 40 min. Factors and their levels in BBD experimental design are shown in Table 4.

BBD was studied to get the higher yield (Y1) and lower crystal size (Y2) of paracetamol crystals produced under USAC. Table 5 and Table 6 represent the Analysis of variance (ANOVA) results for the percentage yield (Y1) and particle size (Y2).

The regression analysis of responses Y1 and Y2 was carried out by backward method for the p-value of less than 0.05, the quadratic model is suggested for response Y1 and the cubic model is suggested for response

Table 4. Box-Behnken design with the observed responses Y1 and Y2.

Experimental Run	A Supersaturation	B Amplitude, %	C Sonication time, min	Y1 Yield, %	Y2 Particle size, volume weight mean, μm
1	1.3	20	20	25.5	13.678
2	1.5	20	20	32.8	14.105
3	1.3	40	20	29.5	13.226
4	1.5	40	20	36.5	10.793
5	1.3	30	10	25.8	14.703
6	1.5	30	10	32.8	13.229
7	1.3	30	30	27.3	16.587
8	1.5	30	30	34.3	12.569
9	1.4	20	10	27.8	13.728
10	1.4	40	10	31.8	14.547
11	1.4	20	30	29.3	13.136
12	1.4	40	30	33.3	14.033
13	1.4	30	20	30.5	15.352
14	1.4	30	20	30.7	15.334
15	1.4	30	20	30.5	15.215
16	1.4	30	20	30.4	15.463
17	1.4	30	20	30.5	15.349

Table 5. ANOVA results of reduced quadratic model for percentage yield (Y1).

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	137.53	5	27.51	3145.61	< 0.0001	significant
A - Supersaturation	100.11	1	100.11	11449.1	< 0.0001	
B - Amplitude, %	30.81	1	30.81	3523.69	< 0.0001	
C - Sonication time,min	4.5	1	4.5	514.64	< 0.0001	
B^2	1.18	1	1.18	135.1	< 0.0001	
C^2	1.04	1	1.04	118.82	< 0.0001	
Residual	0.0962	11	0.0087			
Lack of fit	0.0482	7	0.0069	0.5736	0.7556	not significant
Pure error	0.048	4	0.012			
Cor total	137.62	16				

Y2. However, regression coefficient (R^2) values were observed to be 0.9993 and 0.9980 for responses Y1 and Y2 respectively. Fig. 7 shows the 3D response surface plots for the effect of supersaturation level (factor A), amplitude (factor B), and sonication time (factor C) on the particle size of paracetamol crystal. Based on the ANOVA data's sum of squares, F-value, and P-value, the most influential parameter is determined. The terms in the Model are considered significant if the P-value is less than 0.0500. Variables A, B, C, B^2, and C^2 are important terms for the model in this scenario. The F-value for the lack of fit of 0.5736 shows that the lack of fit for the model is not significant in comparison with the pure error. A significant Lack of Fit F-value has a 75.56 % chance of being caused by noise. A minor fit difference is advantageous. Factors A, B, and C were found to be significant parameters to achieve maximum yield and minimum particle size of crystallized paracetamol

Table 6. ANOVA results of reduced cubic model for particle size (Y2).

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	30.39	10	3.04	306.51	< 0.0001	significant
A- Supersaturation	7.54	1	7.54	760.6	< 0.0001	
B- Amplitude, %	0.7362	1	0.7362	74.26	0.0001	
C- Sonication time, min	0.3058	1	0.3058	30.85	0.0014	
AB	2.04	1	2.04	206.27	< 0.0001	
AC	1.62	1	1.62	163.2	< 0.0001	
A^2	4.18	1	4.18	421.44	< 0.0001	
B^2	8.34	1	8.34	841.63	< 0.0001	
A^2B	3.75	1	3.75	378.64	< 0.0001	
A^2C	0.6786	1	0.6786	68.45	0.0002	
AB^2	1.52	1	1.52	153.22	< 0.0001	
Residual	0.0595	6	0.0099			
Lack of fit	0.0285	2	0.0143	1.84	0.2713	not significant
Pure error	0.031	4	0.0077			
Cor total	30.45	16				

in USAC. The optimum condition was found to be supersaturation level at 1.5, amplitude at 39.99 %, and sonication time at 19.47 min for the maximization and minimization of response Y1 and Y2 respectively which is approximately similar to the experimental condition of run 4 in Table 4.

By analysing the data from Table 5 and Table 6, the model is significant, as indicated by the model's F-value of 306.51. An F-value this large might happen owing to noise only 0.01 % of the time. If the P-value is less than 0.0500, the Model terms are measured as significant. If the value is higher than 0.1000, Model terms are measured as not significant. For the lack of fit, if the F-value is 1.84, it shows that the lack of fit is not significant compared to the pure error. A large Lack of Fit F-value has a 27.13 % likelihood of being caused by noise. Non-significant lack of fit is good.

The following second-order quadratic equation (Eq. 1) is suggested for the response Y1 and the cubic equation (Eq. 2) is suggested for the response Y2 in terms of coded factors.

$$Y1 = 30.53 + 3.54A + 1.96B + 0.7500C + 0.5289B^2 - - 0.4961C^2 \quad (1)$$

$$Y2 = 15.31 - 1.37A + 0.4290B - 0.2765C - - 0.7150AB - 0.6360AC - 0.9948A^2 - - 1.41B^2 - 1.37A^2B + 0.5825A^2C + 0.8715AB^2 \quad (2)$$

where Y1 = percentage yield, Y2 = particle size, A = supersaturation, B = amplitude, and C = sonication time. The R^2-adjusted value obtained for percentage yield is 0.9990, and for particle size, it is 0.9948, which attests to the model's precision and prognostication power.

Characterization

Particle size distribution of raw and USAC paracetamol

Fig. 8 represents the crystal size distribution (CSD) of raw, crystallized paracetamol crystals obtained under USAC at 20°C, 1 h, and 1.5 supersaturation (USAC PCM), BBD experimental run 4. It was observed that raw paracetamol crystal size was in the range of 2.94 to 96.02 µm, with a volume-weighted mean of 18.277 µm and paracetamol crystals produced under USAC, the size of the crystals was in the range from 3.53 to 61.14 µm, with a volume-weighted mean of 18.117 µm. In the paracetamol crystals produced under BBD experimental run 4, the size of the crystals was in the range from 3.67 to 33.43 µm, with a volume-weighted mean of 10.793 µm. CSD of the crystals of paracetamol obtained by USAC was observed to be narrower and uniform in comparison to the CSD of raw paracetamol indicating that the crystals of paracetamol when crystallized in the

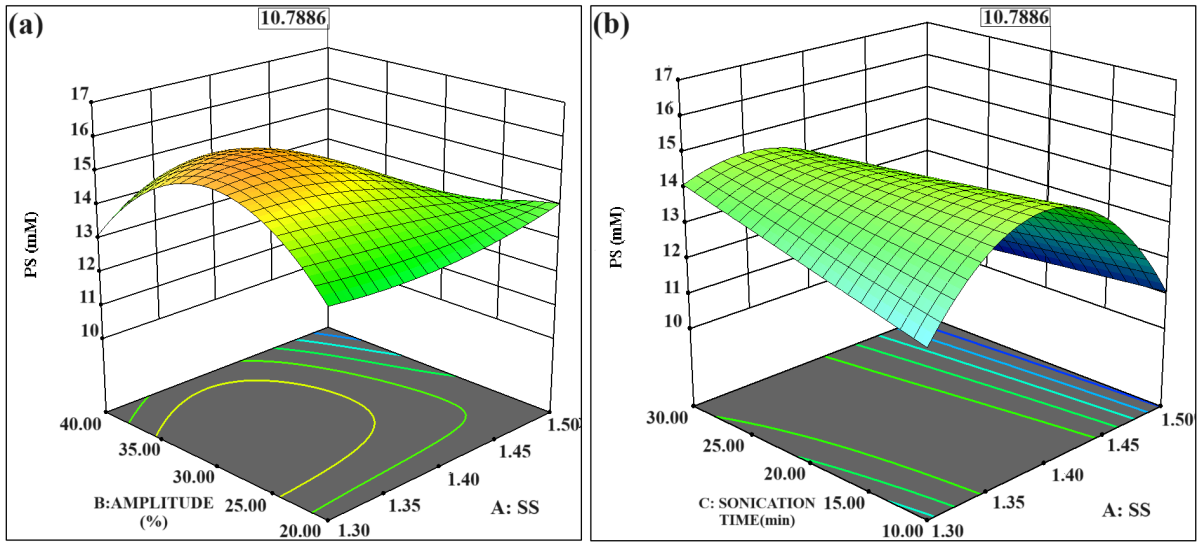

Fig. 7. 3D response surface plots for particle size of PCM as a function of (a) supersaturation (b) amplitude (c) sonication time.

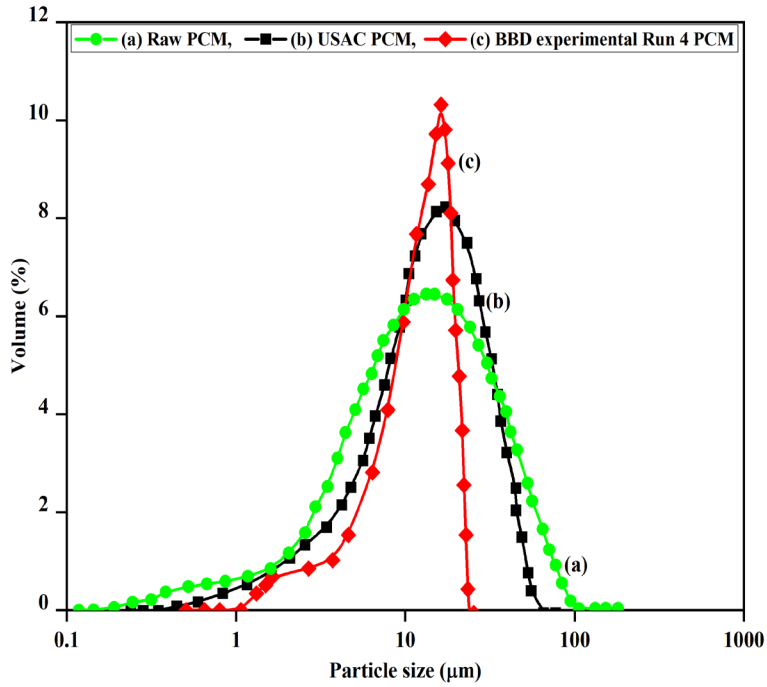

Fig. 8. Particle size analysis for (a) raw paracetamol, (b) crystallized paracetamol under USAC at 20°C, 1 h, and 1.5 supersaturation, and (c) Optimized paracetamol, BBD experimental run 4.

presence of ultrasound [27].

DSC and XRD of raw and USAC paracetamol

The melting and re-crystallization behaviour of paracetamol crystal was also investigated through DSC. Fig. 9 shows the DSC curves of raw paracetamol and the crystallized paracetamol crystals obtained under USAC. DSC of the raw PCM showed endothermic transition at 172.21°C and the USAC PCM showed endothermic transition at 171.93°C [28].

From the XRD analysis, it is clear that the

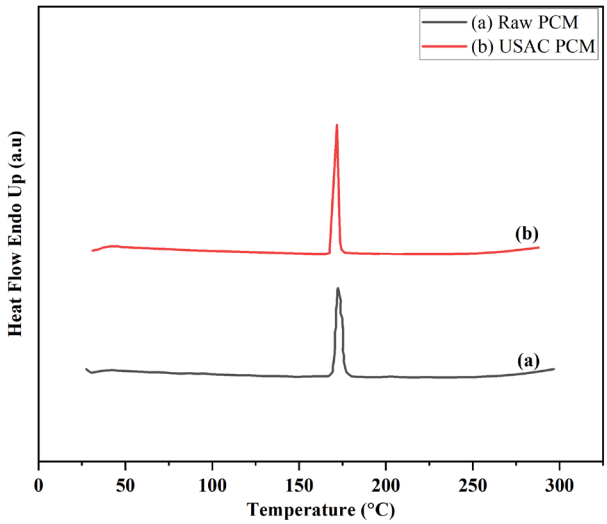

Fig. 9. DSC for (a) raw paracetamol, (b) USAC Paracetamol at 20°C, 1 h, and 1.5 supersaturation.

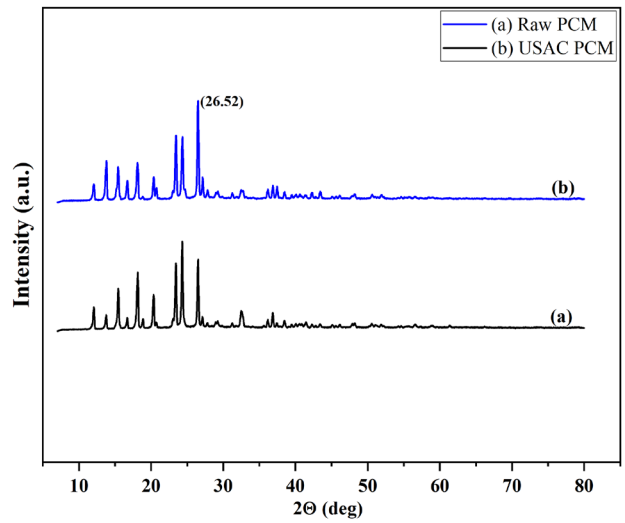

Fig. 10. XRD analysis of raw and crystallized paracetamol: (a) raw paracetamol, and (b) USAC at 20°C, 1 h, and 1.5 supersaturation.

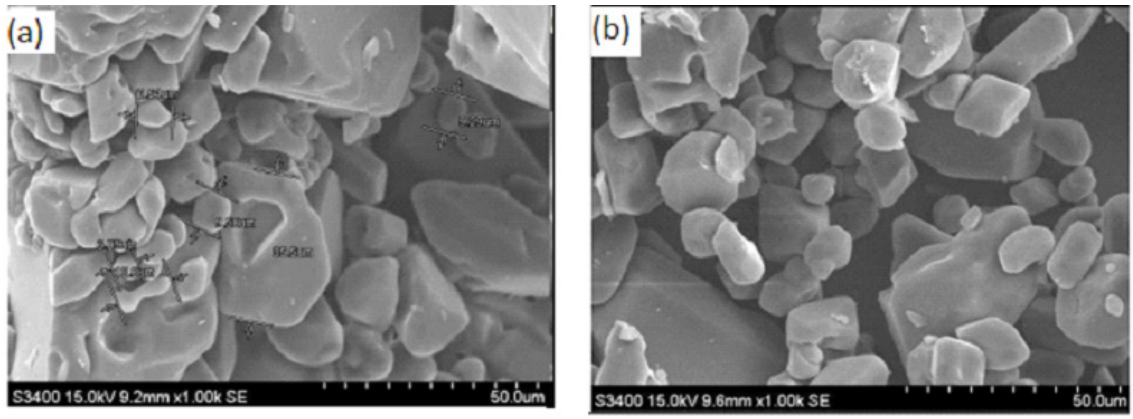

Fig. 11. SEM micrographs of raw and crystallized paracetamol: (a) raw paracetamol (b) USAC paracetamol at 20°C, 1 h, and 1.5 supersaturation.

intensity of USAC crystals obtained maximum at 2Θ of 26.52 leading to higher crystalline characteristics of paracetamol [14] crystals as compared to raw paracetamol crystals as shown in Fig. 10.

SEM of raw and USAC paracetamol

Fig. 11 shows the morphological parameters of raw paracetamol and crystallized paracetamol obtained using USAC at 20°C, 1 h, and 1.5 supersaturation. From the SEM image it showed that the agglomerates crystals of raw paracetamol whereas the USAC process is noticeable with more accurate prismatic shape crystals. A few tiny particles clinging to big crystals were also observed due to the product fragmentation under the presence of ultrasound during the crystallization process [11, 29].

Dissolution study of raw and USAC paracetamol

For the dissolution study of crystallized paracetamol, a 150 mg tablet was prepared using the tablet formulation composition as mentioned in Table 2. The drug release rate of raw and paracetamol crystallized by USAC at 20°C, 1 h, and 1.5 supersaturation is shown in Fig. 12. It can be revealed that, for a raw drug, 19.946 % of release was observed at 5 min and 81.243 % at 90 min and finally it reached 85.354 % at 120 min. In the case of crystals obtained by USAC, it released at an initial rate of 21.973

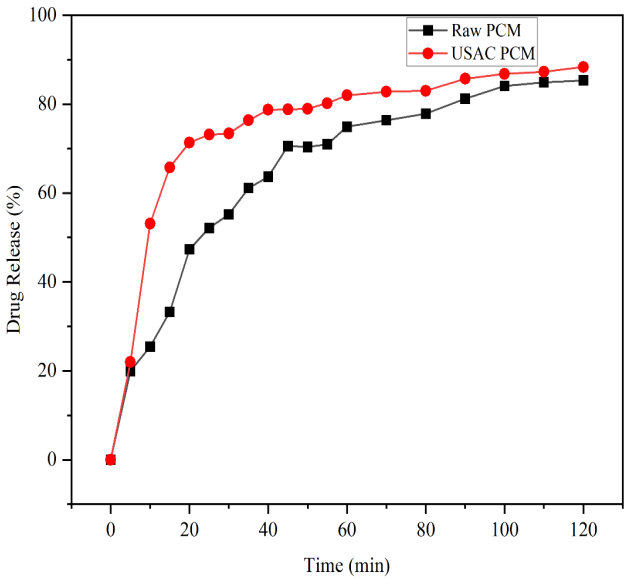

Fig. 12. Drug release profile of raw paracetamol and USAC paracetamol at 20°C, 1 h, and 1.5 supersaturation.

during the first 5 min and it increased to 80.189 % at just 55 min and finally reached 88.346 % of release at 120 min. The dissolution rate data in the current study for a 150 mg tablet showed that USAC enhanced the dissolution rate in comparison to raw paracetamol due to the narrower and uniform crystal size produced. It was found that paracetamol was recrystallized with higher sonication intensity, which resulted in smaller particles and an elliptical crystal with a faster dissolution rate. Also, the study of the effect of ultrasound crystallization of paracetamol along with surface active agent at supersaturation ratio of 1.7 at 30°C sonicated resulted in a reduction of paracetamol crystals size, getting a narrow size distribution and increased dissolution rate [4].

CONCLUSIONS

Ultrasound assisted cooling crystallization (USAC) method was applied to produce the paracetamol crystals. The effect of different parameters such as ultrasound amplitude, supersaturation, temperature, pulsed ultrasound, and crystallization time was studied to improve the percentage yield of the crystals and their size for paracetamol. The use of ultrasound in the cooling crystallization enhanced the supersaturation level and thereby improved the yield and crystal size. It was observed that the supersaturation level to enhance the yield of paracetamol crystals (25.91 %) was achieved in the initial 1h crystallization time and the volume-weighted mean was found to be 18.117 µm for the crystallized paracetamol crystals under USAC at 20°C, 1 h, and 1.5 supersaturation. Further, the optimum condition of BBD was found to be supersaturation level at 1.5, the amplitude at 39.99 %, and sonication time at 19.47 min, and at this condition, the yield of crystals was found to be 36.50 % and the crystal size distribution was found to be uniform and narrower with weighted mean crystal size of 10.788 µm which is similar to the BBD experimental run 4. The morphology of crystallized paracetamol crystals in the USAC process was found more accurate in prismatic and rod-shaped crystals. The drug release profile showed that the crystallized paracetamol crystals released 80.189 % at just 55 min whereas raw paracetamol crystals needed 90 min to release 81.243 % for the 150 mg tablet formulation. Therefore, the pharmaceutical industry can explore the utilization of ultrasound during the cooling crystallization of active pharmaceutical ingredients to tailor the yield and particle size as needed.

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