DESIGN AND OPTIMIZATION OF MICROCRYSTAL PRODUCED BY SONOPRECIPITATION TECHNIQUE: OPTIMIZATION OF PROCESS PARAMETERS

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***Abstract*- The aim of the present work was to prepare microparticles by sonoprecipitation method for targeted drug delivery of Beclometasone dipropionate (BDP), an anti-inflammatory corticosteroid available for the treatment of asthma. Experimental designing employed using Design-Expert 5.0 software (StatEase, QD Consulting, Penzance, UK) with linear as design model. The factors investigated were addition rate, stabilizer conc. and sonication time. The addition rate of solvent i.e acetone was studied at 50(-1), 100(0) and 150(1) g /min, stabilizer conc. were 0.5(1), 1(0) and 1.5 %(1) and sonication time investigated were 1(-1), 2(0) and 3 (1) min. Dry powder were characterized by XRD, DSC, SEM, FTIR, flow property, Particle Size, Particle Distribution and Aerodynamic Particle Size Analysis.It is worth that the particles were uniform in the presence of long time of sonication showed the highest FPF loaded and FPF emitted of 41 (2%) and 84(4%) respectively, depositing mainly on stages 3 and 4, with much lower amounts collected on the higher stages of the MSLI.**

Highlights:

- **Developed spherical microparticles with size range of 1to 5 µm.**
- **Factors investigated were addition rate, stabilizer conc. and sonication time.**
- **Highest Fine Particle Fraction loaded of 41 (2%) and emitted 84(4%) respectively.**

***Keywords* – Beclometasone dipropionate, Sonoprecipitation technique, Central Composite Design, Crystal engineering.**

INTRODUCTION

Inhaled corticosteroids are recognized as the premieretherapeutic agents for the control of persistentasthma.[1] However, the effectiveness of these agents isoften compromised by poor adherence and incorrectinhaler technique that results in inadequate drug deliveryto the lung.[2] Moreover, even with optimal technique,only approximately 10% of steroid aerosols generatedfrom chlorofluorocarbon-based metered doseinhalers reach the lower respiratory tract, and most ofthe medication delivered to the lung is deposited in thelarge central airways.[3] These observations suggest thatconventional inhaled corticosteroids may have limitedeffectiveness in suppressing inflammation in andimproving patency of the small airways in asthma.

Prolonged systemic administration of corticosteroidscan cause many serious adverse effects that can bereduced by aerosol administration.[4]The most frequentlyobserved adverse effects of aerosolized corticosteroidshave been oropharyngeal candidiasis and hoarseness.The importance of a dose-dependent systemic effect iscontroversial.[5]

Beclometasone dipropionate (BDP) is an anti-inflammatory corticosteroid available for the treatment of asthma. BDP was previously developedas aqueous nasal formulations (Beconase AQ, [6]Vancenase AQ) for the treatment of Asthma. BDP wasalso available in dry nasal aerosol formulations aschlorofluoro carbon (CFC) metered-dose inhaler nasalsprays (Beconase and Vancenase Pockethaler),but CFC-containing formulations have been withdrawnfrom the market and currently are not available.[7] BDP hydrofluoroalkane (HFA) inhalationaerosol (QVAR) currently is available for patientuse and indicated for the maintenance treatment ofasthma as prophylactic therapy in patients 5 years ofage and older.[8] BDP HFA nasal aerosol (QNASL),an intranasal aerosol formulation, has been developedrecently for the treatment of asthma.[9]

Sonocrystallisation is sought here as an alternative methodto affect the production of NaCl for pharmaceutical use.Apart from constituting a rapid application, sonocrystallisationalso offers further advantages including smaller crystal sizeproduced compared to conventional crystallisation, cost effectivenessof apparatus, the process can be run at ambientconditions and the reaction vessel involved is of simple geometrymaking the cleaning process simple for the pharmaceuticalrequirements. Another important advantage is that becausecrystal growth occurs at lower supersaturation levels where initialgrowth is controlled, the size distribution of the products isnarrower than uncontrolled crystallisation process.[10-11]

The aim of the present study was to apply ultrasoundduring anti-solvent precipitation for producing discreet and non-agglomerated microparticles for dry powder inhalation. Microparticles werecharacterized by XRD, DSC, SEM, FTIR, flow property, Particle Size, Particle Distribution and Aerodynamic Particle Size Analysis.

2.1. MATERIALS AND METHODS

2.2. Materials.

Beclometasone dipropionate was supplied from GlaxoSmithKline Pharmaceuticals Limited, Mumbai, Maharashtra, India and Acetone and HPMC (A.R. grade) was purchased from Loba Chemie Pvt. Ltd, Mumbai, India.

2.3. Sonoprecipitation

2.3.1. Crystallization Procedure

In this study, the equipment consisting of a probe and sonifier (PCi Analytics, DP120) was used. A probe has a tip diameter of 13 mm and is immersed to middle in the liquid. The device operates at a HighElectricalEnergyoperatingatafrequency of20KhzandanUltrasonicPower 120 W. A 500-ml jacketed glass sonoreactor was used in these experiments providing control over temperature. Unprocessed Beclometasone dipropionate (BDP) was dissolved in acetone and filtered through 0.45 µm pore size membranes to remove the possible impurities. The solution was then poured at a controlled rate using a model 505S peristaltic pump (Watson Marlow Bredel Pumps Ltd., Falmouth, UK) into watercontaining stabilizer which kept in sonoreactor and simultaneously irradiated with varying sonication time.Resultant suspension was freeze dried (Labmate (Asia) Pvt. Ltd.Chennai, India.).

2.2.3 Experimental design for the optimization of crystallization conditions

A response surface type central composite design were employed using Design-Expert 5.0 software (StatEase, QD Consulting, Penzance, UK) with linear as design model. The factors investigated were addition rate, stabilizer conc.and sonication time. The addition rate of solvent i.e acetone was studied at 50(-1), 100(0) and 150(1) g /min, stabilizer conc. were 0.5(1), 1(0) and 1.5 %(1) and sonication time investigated were 1(-1), 2(0) and 3 (1) min,

2.3. Solid-State Characterization.

2.3.1. X-ray diffraction studies

Powder X-ray patterns were recorded for pure drug and freeze dried product using a Bruker AXS diffractometer (Bruker AXS GmbH, Germany) with a PSD-50M detector and EVA Application Software version6. Measurements were performed with a Cu Ka radiation source at 40 kV voltage, 30 mA current and a maximum scanning speed of 2^0/min.

2.3.2. Differential scanning calorimetry

DSC curves were obtained for pure drug and freeze dried product by a Differential Scanning Calorimeter (DSC 821e, Mettler-Toledo, Switzerland) at a heating rate of 5 K/min from 0 to 340^0C under nitrogen.

2.3.3. Scanning Electron Microscopy

Particle morphology was examined by Scanning Electron Microscopy (SEM) (S250MK, Cambridge,U.K.) operating at 19 keV or SEM (JSM-6360LV, JEOL, Japan) operating at 20 keV. The pure drug and freeze dried powder could be characterized as dry powder. Samples were mounted on the copper stub by copper tape and sputter coated with Au at 6 mA for 3 min using a sputter coater (KYKY SBC-12, Beijing, China) under an Ar atmosphere.

2.3.4 Fourier transform infrared (FT-IR) spectroscopy

FT-IR was taken by FT-IR instrument (Perkin Elmer, USA) fordifferent Beclometasone dipropionate samples at scanning range between 450 cm−1 and4000 cm−1. Each sample (several milligrams) was placed in themiddle of the sample stage and a force applied (50 bar) using thetop of the arm of the sample stage. After obtaining sharp peaksof appropriate intensity, the spectra acquired were the results ofaveraging four scans at 1 cm−1.

2.3.5 Powder flow characterization

Carr's index (CI) and angle of repose (α) were measured for freeze dried Beclometasone dipropionate powders as an indication of powder flow ability. Each powderwas filled into a 5 mL measuring cylinder and after recordingthe volume (bulk volume) the cylinder was tapped 100 times underambient conditions (20 ◦C, 50% RH) and the new volume (tap volume)was recorded. It has been observed that 100 taps was sufficient to attain the minimum volume of the freeze dried Beclometasone dipropionate powders bed. Then, bulk density (Db), tap density (Dt), Carr's Index (CI), and porosity for each freeze dried Beclometasone dipropionate powders were calculated. The powders were subjected to bulk density and tapped density determination using Tapped density tester USP II (Veego, India).Angle of repose was measured by the method adapted from(Kaialy et al., 2011a). In brief, a pile was built by dropping 1 g ofeach through a 75 mm flask on a flat surface. Angle of repose (α)was calculated using the following equation (where h is the heightof the powder cone, and D the diameter of the base of the formedpowder pile).

$\tan \alpha = 2h/D$……(1)

2.3.6 Particle size and distribution determination

Particle sizing was carried out by laser diffraction using the Malvern MastersizerX (Malvern Instruments Ltd.,Malvern,UK) equipped with a 100 mm focal length lens and anMS7 magnetically stirred cell using the 2 NHE software presentation. The sizing methods were validated according to ISO 13320 (1990) standards. The freeze dried Beclometasone dipropionate powders (approximately 1 mg) were added to an 8 mL glass vial and 2 mL of filtered dispersant was added. The suspension was sonicated (BMI 599, Biomedica) in a water bath for 5 min to allow dispersion of the particles and aliquots were added successively to the sample cell by means of a Gilson pipette to achieve a satisfactory obscuration level (20% obscuration) and allowed to equilibrate for 60 s. Mean Particle size and Particle size distribution measurements were taken with each batch.

2.3.7. Aerodynamic particle size analysis

The dispersion behaviour of the freeze dried Beclometasone dipropionate powders were assessed using an Aerolizer (Novartis Pharmaceutical, Australia) as the inhaler coupled through a USP stainless steel throat to a Multistage Liquid Impinger (MSLI, Copley, U.K.), operating at 60 L/min controlled by the flow meter (DMF2000, Copley, U.K.) for 4 s. The MSLI is a versatile five-stage cascade impactor which can be used for determining the *D*ae distribution of Dry Powder Inhalation (DPI). The design is such that at a flow rate of 60L/min, the cut off diameters of stages 1, 2, 3, and 4 are 13, 6.8, 3.1, and 1.7 μm, respectively. Stage 5 comprises an integral paper filter to capture the remaining fraction of particles less than 1.7 μm. By evaluating the DPI, it is then possible to calculate the mass fraction of drug particles smaller than 5 μ min the aerosol cloud, i.e., fine particle fraction (FPF). For MSLI, the FPF loaded and FPF emitted are defined as follows.

$$\text{FPF loaded} = \frac{\text{powder mass on stage 3 + stage 4 + filter}}{\text{total mass recovered}} \qquad (2)$$

$$\text{FPF emited} = \frac{\text{powder mass on stage 3 + stage 4 + filter}}{\text{total mass recovered} - \text{capsule and device retention}} \quad (3)$$

Where the capsule and device retention is the mass of drug remaining in the capsules and the device. Stages 1-4 of MSLI were injected into 20.0 mL of methanol via a 0-5000 μL pipet (Transforpette, Brand, Germany), and the filter stage of MSLI is covered with a GF/A filter paper (70mm, Whatman, U.K.). The powder (10.00 (0.50 mg) was filled into Vcaps capsules (size 3, Capsuge, Suzhou, China). After analysis, the capsule, inhaler, throat, and filter paper were carefully rinsed with 20 mL of acetone. The MSLI itself was tightly sealed with Parafilm (Pechiney Plastic Packaging, USA) to avoid evaporation losses and gently shaken for 5-10 min to dissolve drug from each stage. Each experiment was performed in duplicate. Freeze dried Beclometasone dipropionate deposited at different locations was determined by UV-spectrophotometer at 238 nm (UV-2501PC,Shimadzu).

3. RESULTS AND DISCUSSION

3.2 Physico-chemical characterization

3.2.1 X-ray diffraction studies

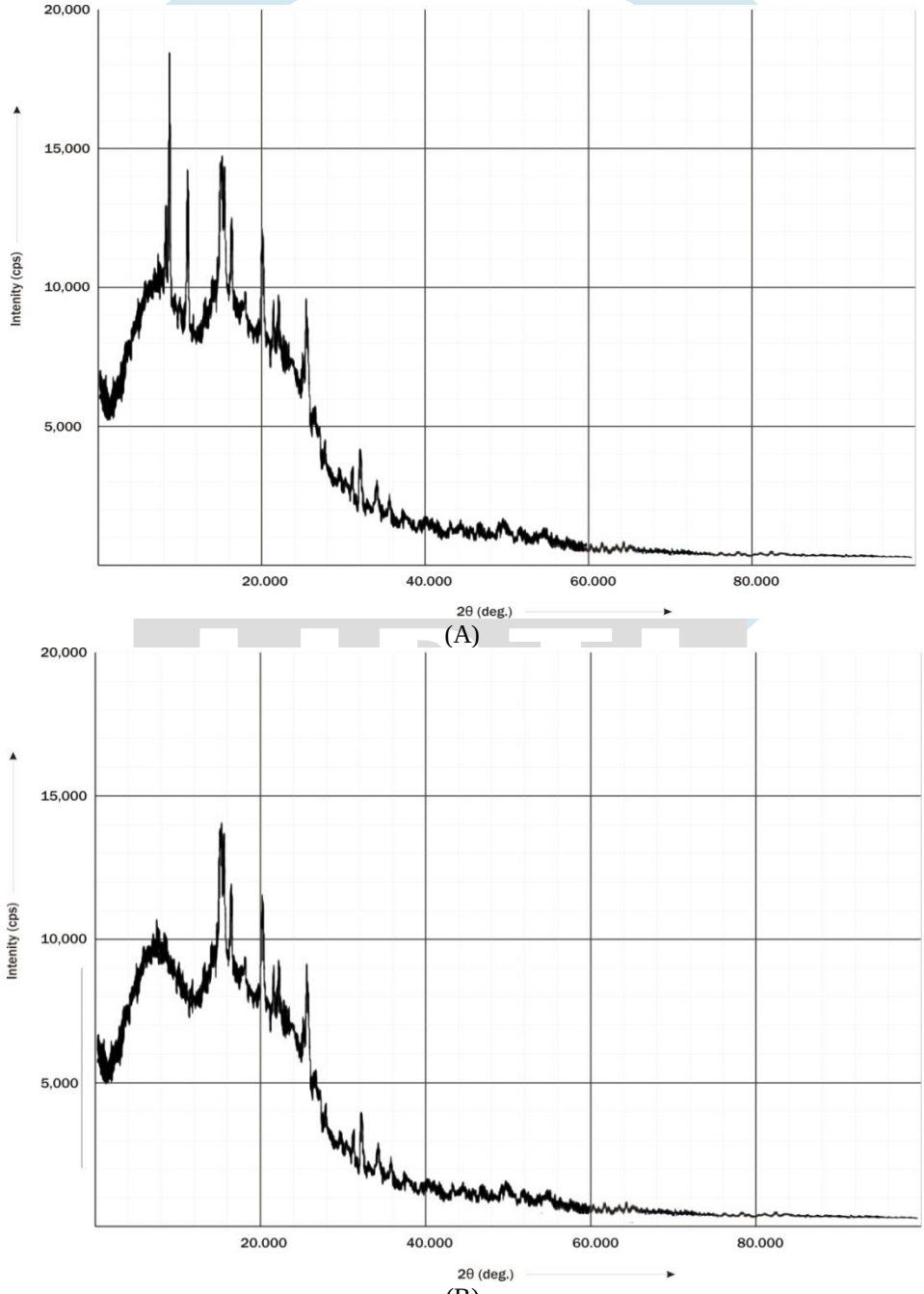


Figure 1.X-ray diffraction patterns of various Beclometasone dipropionate dry powders: (A) commercial Beclometasone dipropionate product; (B) freeze dried product.

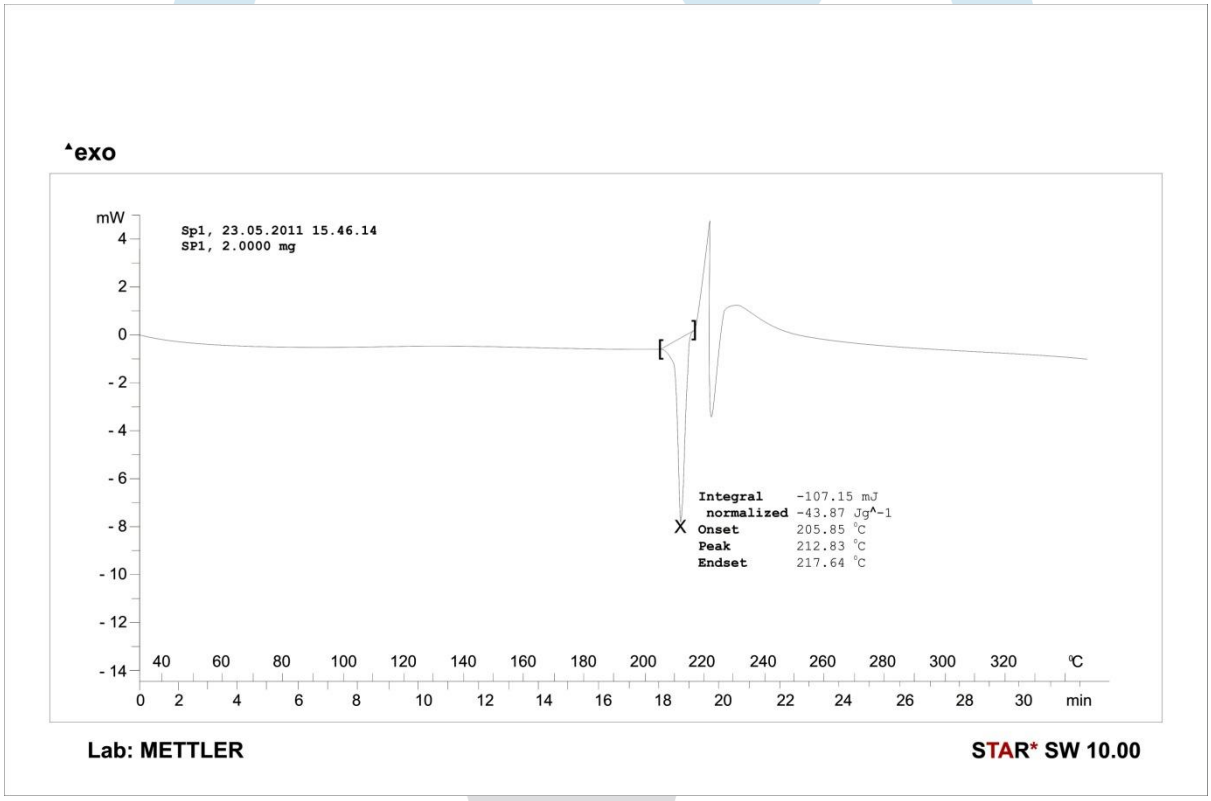
Diffraction techniques are definitive tools used for detecting and quantifying molecular order in a system. The PXRD analysis of the anhydrous sample (drug) showed a typical Beclometasonedipropionate diffraction pattern which includes strong peaks at 10, 12, 15 and 20 2θ values (figure 1).[12] Two of the strongest peaks in the freeze dried Beclometasonedipropionate 2θ values of 8 and 12 were not present in the original crystalline material. These peaks can explain presence of BDP monohydrate i.e. BDP.H_2O. These peaks are more prominent when there is long contact time of BDP with water.[13]

Although no evidence of pure solvate formation to be gained from this qualitative data alone, it does demonstrate that the crystalline material that was generated from the freeze dried product has different solid-state properties compared to the pure drug. BDP can form several crystal structures that include solvates and polymorphs. The new diffraction pattern of freeze dried product was characterized by strong diffraction peaks, and represents a new polymorph of Beclometasone dipropionate.[14-15]

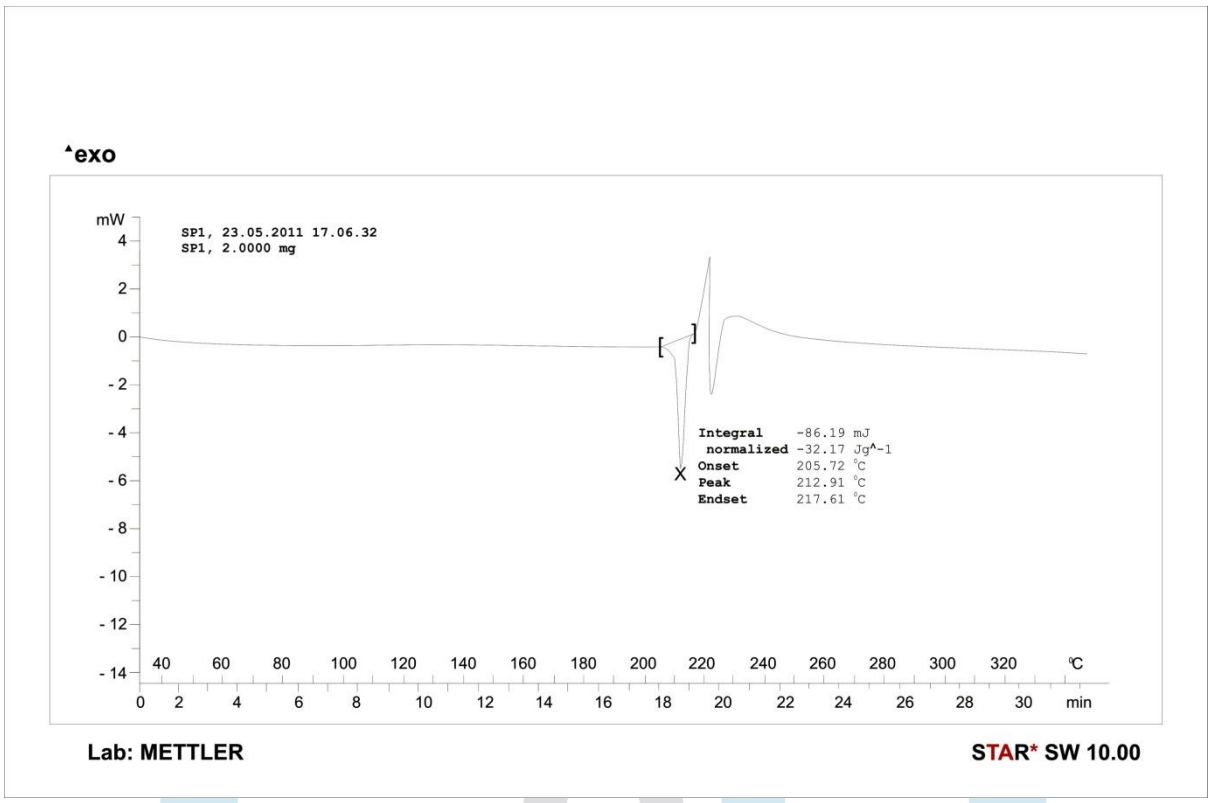
3.2.2 Differential scanning calorimetry

DSC is mainly used for investigating the phase behavior of pharmaceutical solids and different methodologies based on instrument type can be used. Conventional DSC, as used here, is based on a linear heating rate of the sample through to its melting point to degradation. Anhydrous BDP (drug) displayed a single endothermic transition onset peak at 208°C, which corresponds to melting transition (Tm) of the steroids (Figure 2; previously reported as 213°C). The absence of an exothermic re-crystallization transition or a glass transition (Tg) suggests that this material was predominantly crystalline and contained little or no amorphous content.

But there is slight decrease in enthalpy. These decreases may be caused from three factors: 1) As fragmented particles' surface/volume ratio is increased, less energy is needed for melting; 2) Impurity may be present in fragmented particles; 3) Part of the particles may be amorphous. The complex thermal events around the melting point observed in the different DSC curves of the different samples indicate that the BDP undergoes significant degradation during melting.[16]



(A)



(B)

Figure 2. The DSC thermograms of Beclometasone dipropionate (A) and freeze dried powder (B).

3.2.3 Scanning Electron Microscopy

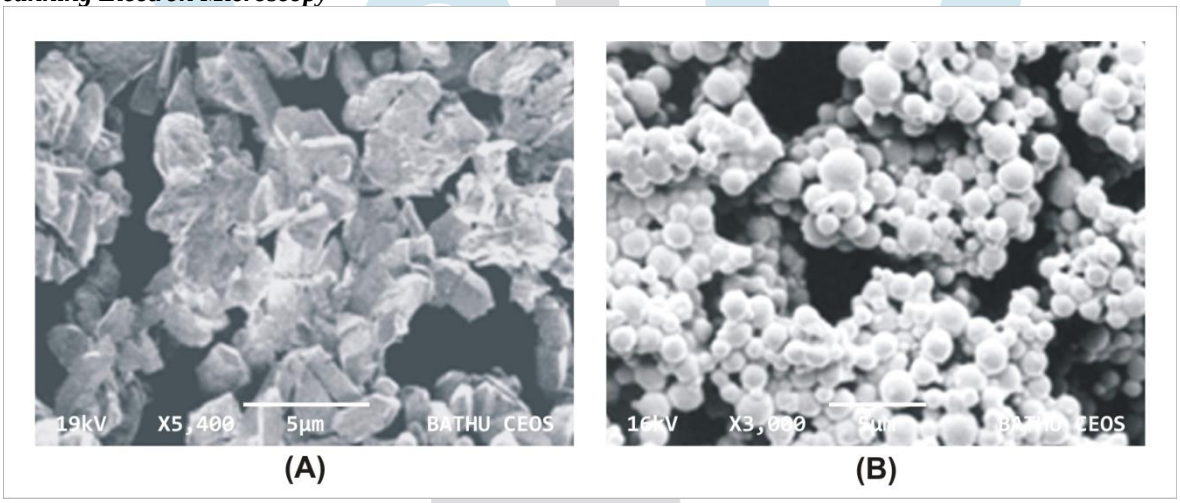


Figure 3.SEM photographs of various Beclometasone dipropionate particles: (A) Pure drug; (B) Freeze dried powder.

The untreated Beclometasone dipropionate crystals are observed in Fig. 3A using electron microscopy (300×) and microcrystals of are shown in Fig. 3B (5000×). The micrographs show that microcrystals were spherical with smooth and about 1–9 µm in size while the mean diameter of the pure drug is about 137 µm $\pm$ 6 and the crystals were rod-shaped. Whereas the powder produce by sonoprecipitation technique is free from agglomerates this can be demonstrated by the occurrence of recrystallization at the proper contact point between particles.[17-18]

3.2.4 Fourier transform infrared (FT-IR) spectroscopy

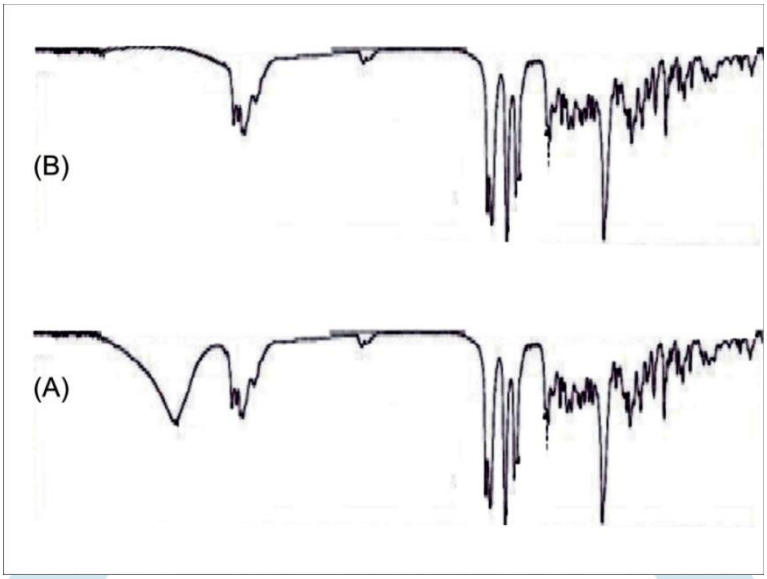


Figure 4.Fourier Transform Infrared Spectroscopy (FT-IR) of various Beclometasone dipropionateparticles: (a) Freez dried product; (b)Pure drug.

FTIR spectrogram(Fig.4) shows no changes in the fingerprint region (500-1800 cm-1), but new peaks are observed at 3500-3600 cm-1 (OH band) in all BDP samples in aqueous suspension. This modification corresponds to theFTIR spectra of BDP monohydrate. BDP is transformed into BDP.H_2O due to its contact with water. The transformation does not affect the therapeutic properties of thedrug, as both anhydrous BDP and BDP.H_2O are commercialized anti-asthmatic drugs.

3.3 Powder flow characterisation

True density (D_{true}), bulk density (D_b), tap density (D_t), Carr's index (CI), angle of repose (α), and porosity for all formulations were calculated. All formulations showed identical D_{true}(0.47± 0.21 g/cm^3) as comparison with pure drug (D_{true}(0.69± 0.35 g/cm^3)) it shows lesser value for are attributedto its different crystalline nature. All formulations showed dissimilar D_b, D_t, CI, α and porosity. Direct linear proportionality (r2 = 0.961) was found between CI and α showing good agreement between the two methods. Both CI and α data indicated good flow character of all formulations, which is needed to achieve satisfactory DPI formulation. By comparing different formulations with pure drug powder, formulations showed the lowest D_b (0.38± 0.14 g/cm^3), the lowest D_t (0.26±0.47 g/cm^3), and the highest porosity (86.2±0.48%) whereas pure drug powder showed the highest D_b (1.46±0.41 g/cm^3), the highest D_t (1.82±0.21 g/cm^3), and the lowest porosity (32.4±0.74%). This indicates that, contrary to pure drug powder, formulations have the lowest cohesiveness properties and lowest interparticulate forces. This could be attributed to spherical particle shape in case of formulations, which is likely to lead to increased void space between particles.

3.4 Response surface construction to optimize crystallization parameters

The mean particle size and Fine Particle Fraction (FPF) of BDP obtained by using sonoprecipitation technique was studied. There is also brief elaboration of effect of Antisolvent Addition Rate (ml/min), Stabilizer conc. (%) & Sonication time (min) on crystal growth. For the optimization purpose we select Central Composite Design (Table 1&2) with linear and Quadratic as design model from mean particle size and Fine Particle Fraction (FPF) respectively for response surface category (Table 2). It gives 20 runs, with no blocks in the design. Three factors i.e. Antisolvent Addition Rate (ml/min), Stabilizer conc. (%) & Sonication time (min) to be considered as independable variables. Response for mean particle size (Dependable Variable 1) and Fine Particle Fraction (FPF) (Dependable Variable 2) was analyzed with polynomial. Dependable Variable 1 ranges from 2.300 to 13.500 and for dependable Variable 2 it was from 41 to 84. Current design models do not required data transformation.

Table 1. Experimental runs according to Central composite design with their coded values.

Standard	Runs	A: Addition rate	B: Stabilizer conc.	C: Sonication time	Mean Particle Size	Fine Particle Fraction (FPF)
2	1	1.00	-1.00	-1.00	11.5	52
7	2	-1.00	1.00	1.00	3.7	75
8	3	1.00	1.00	1.00	2.3	84
16	4	0.00	0.00	0.00	7.4	67
4	5	1.00	1.00	-1.00	6.2	43
17	6	0.00	0.00	0.00	7.4	67
11	7	0.00	-1.68	0.00	5.8	56
1	8	-1.00	-1.00	-1.00	13.5	41
19	9	0.00	0.00	0.00	7.4	67
6	10	1.00	-1.00	1.00	6.9	74

20	11	0.00	0.00	0.00	7.4	67
10	12	1.68	0.00	0.00	4.7	51
15	13	0.00	0.00	0.00	7.4	67
14	14	0.00	0.00	1.68	2.4	78
3	15	-1.00	1.00	-1.00	5.2	53
5	16	-1.00	-1.00	1.00	3.4	57
18	17	0.00	0.00	0.00	7.4	67
13	18	0.00	0.00	-1.68	6.8	55
12	19	0.00	1.68	0.00	3.5	68
9	20	-1.68	0.00	0.00	9.3	48

Table 2. Design Summary for 30 runs with quadratic and 2FI as design Models.

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std. Dev.
A	Adition rate	ml/min	Numeric	-1.00	1.00	-1.000	1.000	0.000	0.826
B	Stabilizer conc.	%	Numeric	-1.00	1.00	-1.000	1.000	0.000	0.826
C	Sonication Time	min	Numeric	-1.00	1.00	-1.000	1.000	0.000	0.826

Response	Name	Analysis	Min.	Max.	Mean	Std. Dev.	Ratio	Tran.	Model
Y1	Mean Particle Size	Polynomial	2.300	13.500	6.480	2.769	5.870	None	Linear
Y2	Fine Particle Fraction	Polynomial	41.000	84.000	61.850	11.508	2.049	None	Quadratic

4.5.1.1 Mean Particle size

Correlation of addition rate was -0.145, stabilizer concentration was -0.476 and sonication time was -0.601, with Mean Particle size (Dependable variable 1) shown in Figure 5, Figure 6 and Figure 7 respectively. Addition rate, stabilizer conc., and sonication time shows negative correlation. From this it was conclude that there was negative effect of addition rate, stabilizer conc., and sonication time on the mean particle size.

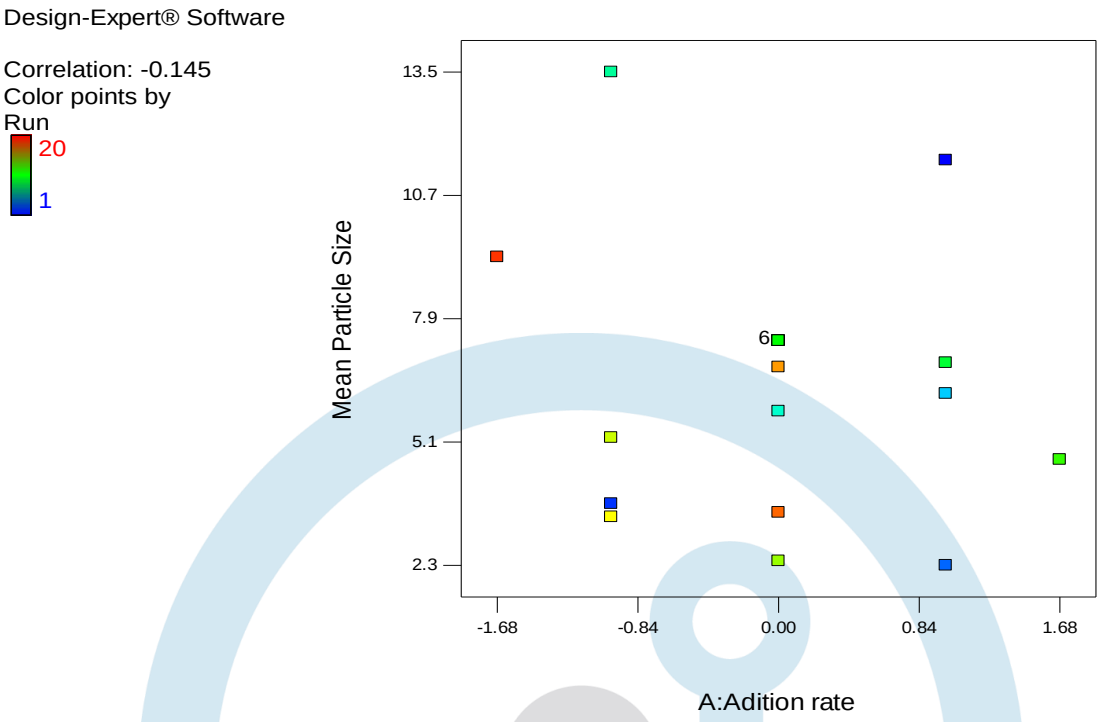


Figure 5.Correlation between Addition rate and Mean particle size.

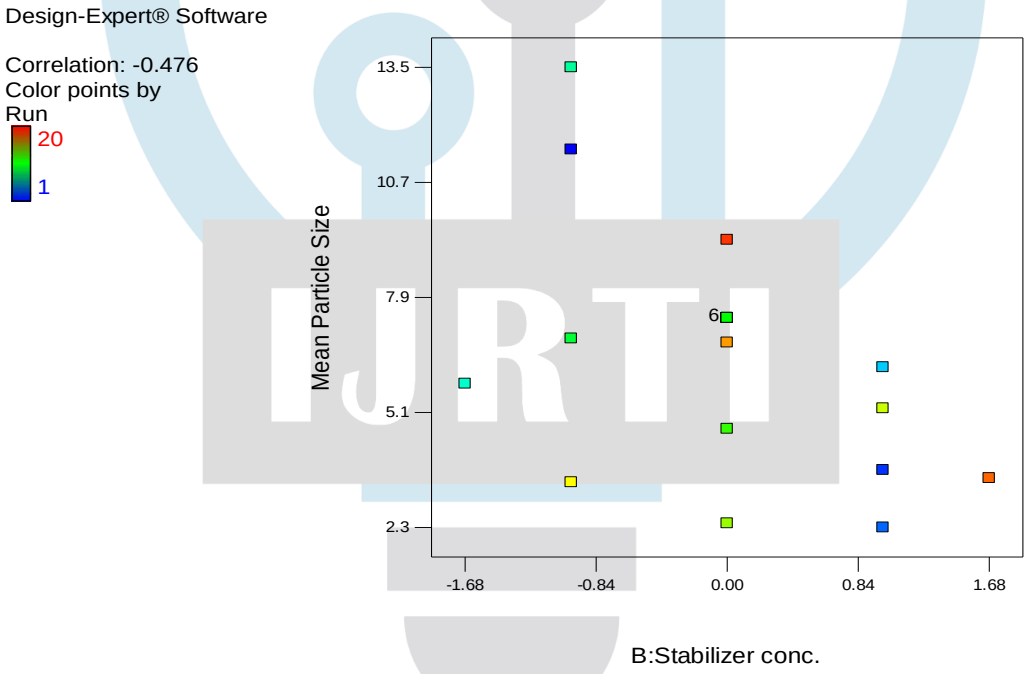


Figure 6.Correlation between Stabilizer conc. and Mean particle size.

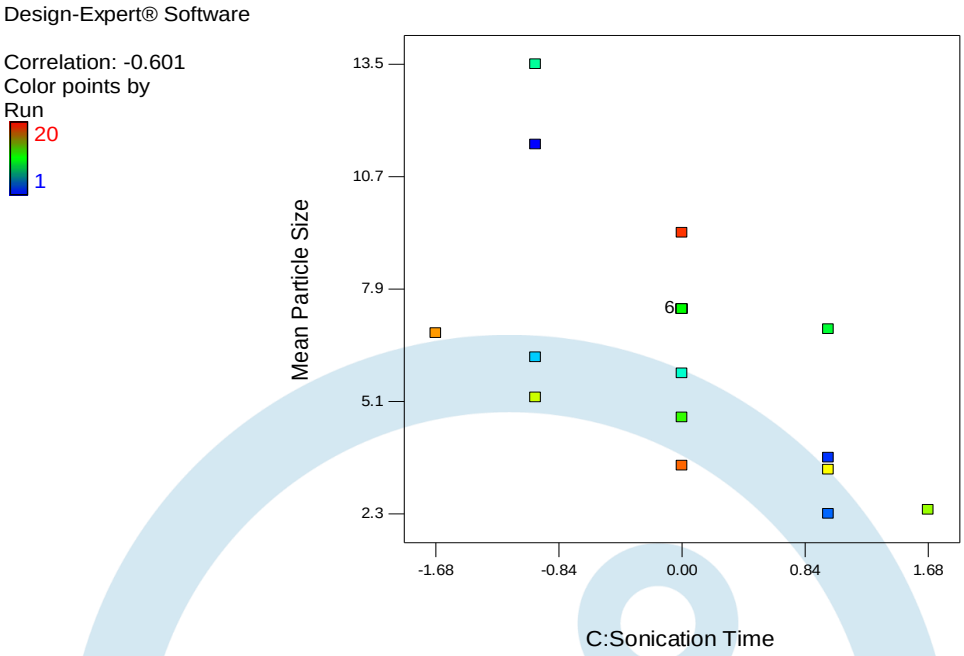


Figure 7.Correlation between Sonication time and Mean particle size.

The cubic and quadratic model was aliased. This means not enough experiments have been run to independently estimate all the terms for this model. The suggested model is Linear (Table 3 and 4).

Table 3. Sequential Model Sum of Squares [Type I] for mean particle size

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	839.81	1	839.81			
Linear vs Mean	93.30	3	31.10	8.29	0.0015	Suggested
2FI vs Linear	12.46	3	4.15	1.13	0.3714	
Quadratic vs 2FI	14.15	3	4.72	1.41	0.2964	
Cubic vs Quadratic	28.11	4	7.03	7.92	0.0142	Aliased
Residual	5.32	6	0.89			
Total	993.16	20	49.66			

Table 4.Model Summary Statistics for mean particle size.

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.94	0.6084	0.5350	0.3019	107.05	Suggested
2FI	1.91	0.6897	0.5464	-0.1886	182.27	
Quadratic	1.83	0.7820	0.5857	-0.7347	266.03	
Cubic	0.94	0.9653	0.8901	-6.6531	1173.62	Aliased

ANOVA (Table 5) suggested for the design is as follows. The Model F-value of 8.29 implies the model is significant. There is only a 0.15% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms.

Table 5.ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares - Type III]for mean particle size.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	93.30	3	31.10	8.29	0.0015	significant
A-Adition rate	*3.22*	*1*	*3.22*	*0.86*	*0.3678*	
B-Stabilizer conc.	*34.70*	*1*	*34.70*	*9.24*	*0.0078*	

C-Sonication Time	*55.37*	*1*	*55.37*	*14.75*	*0.0014*	
Residual	60.06	16	3.75			
Lack of Fit	*60.06*	*11*	*5.46*			
Pure Error	*0.000*	*5*	*0.000*			
Cor Total	153.35	19				

Final Equation in Terms of Coded Factors:

Mean Particle Size =

+6.48

-0.49 * A

-1.59 * B

-2.01 * C……(4)

Final Equation in Terms of Actual Factors:

Mean Particle Size =

+6.48000

-0.48593 * Adition rate

-1.59393 * Stabilizer conc.

-2.01363 * Sonication Time……..(5)

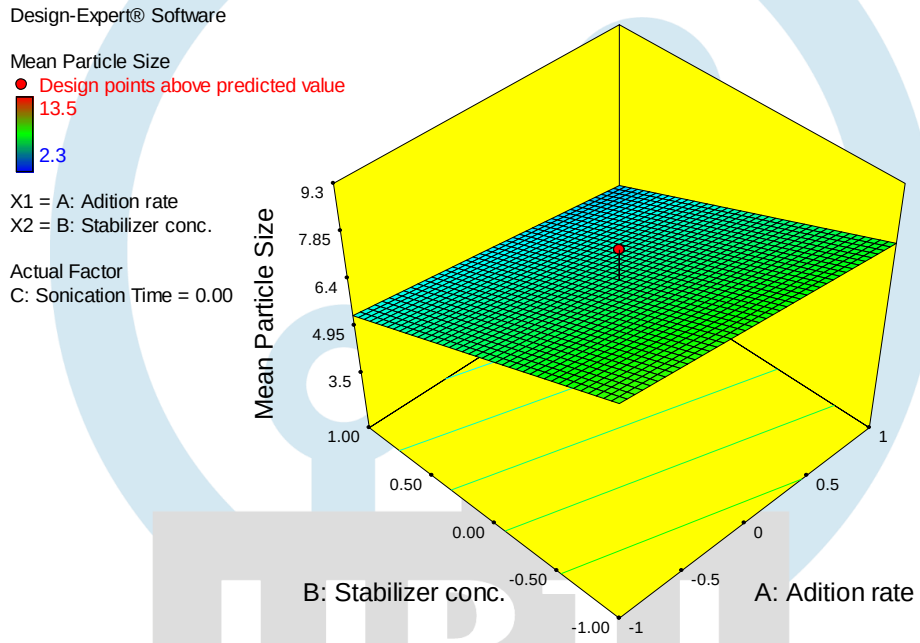


Figure 8.Response surface graph for Mean particle size with respect to Stabilizer conc. and Addition rate as prominent factors.

The size distribution of BDP was shown in Figure 8. The crystals were the largest where there is less amount of stabilizer concentration and lesser sonication time. Statistical analysis of the particle size of microcrystal's produced by different formulations by a two-way ANOVA test showed that the concentration of the stabilizer and sonication time had a significant effect on the particle size of the microcrystal's while the addition rate did not have a significant effect on the particle size.

4.5.1.2 *Fine Particle Fraction (FPF)*

Correlation of Addition rate was 0.168, Stabilizer concentration was 0.269 and sonication time was 0.734 with fine particle fraction (Dependable variable 2) shown in Figure 9, Figure 10.and Figure 11.respectively.

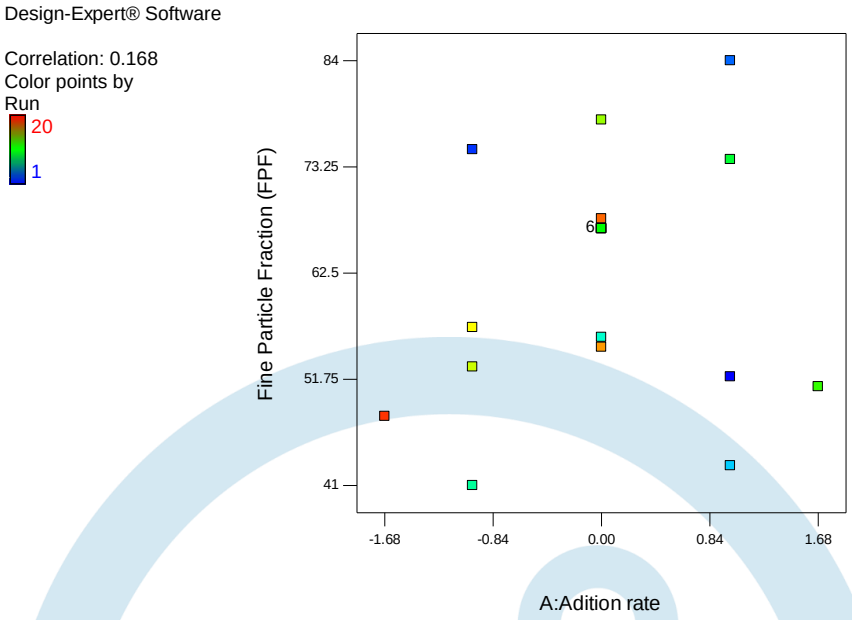


Figure 9.Correlation between Addition rate and fine particle fraction.

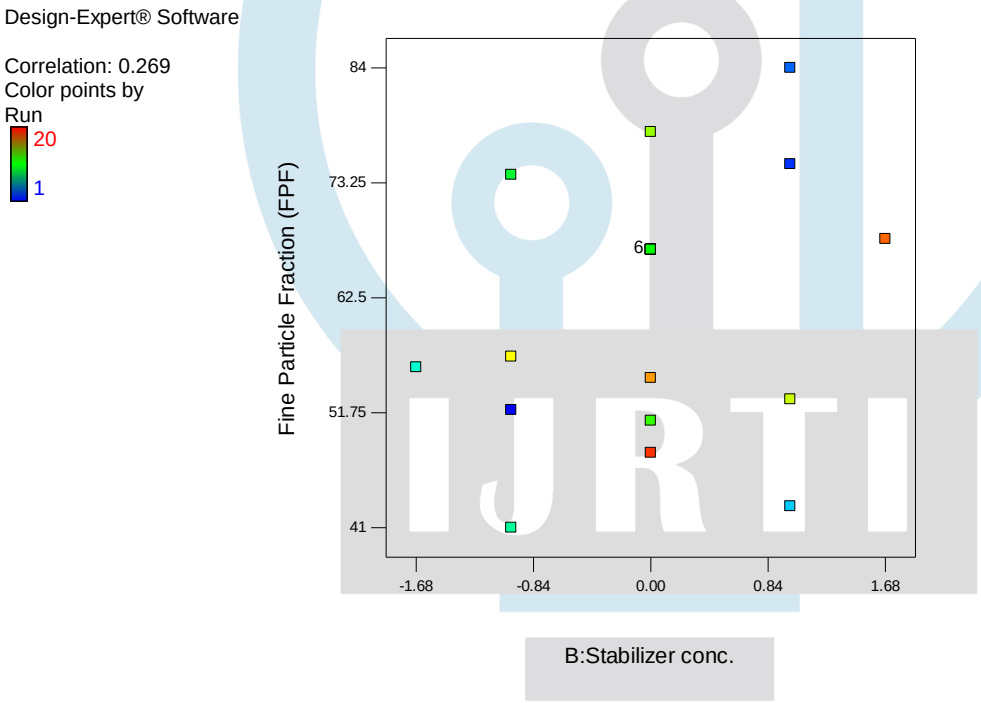


Figure 10.Correlation between stabilizer conc. and fine particle fraction.

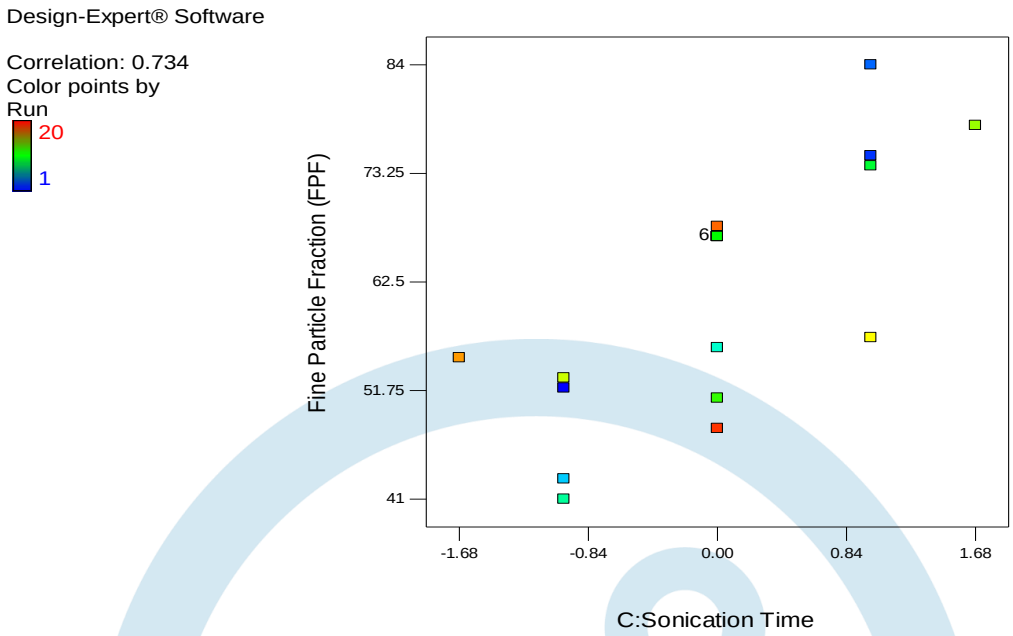


Figure 11.Correlation between sonication time and fine particle fraction.

The cubic model is aliased. This means not enough experiments have been run independently estimate all the terms for this model. The suggested model is Quadratic (Table 6 and 7).

Table 6. Sequential Model Sum of Squares [Type I] for fine particle fraction

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Mean vs Total	76508.45	1	76508.45			
Linear vs Mean	1695.65	3	565.22	9.49	0.0008	
2FI vs Linear	261.38	3	87.13	1.64	0.2290	
Quadratic vs 2FI	535.44	3	178.48	11.43	0.0014	Suggested
Cubic vs Quadratic	152.85	4	38.21	70.83	< 0.0001	Aliased
Residual	3.24	6	0.54			
Total	79157.00	20	3957.85			

Table 7.Model Summary Statistics for fine particle fraction.

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	7.72	0.6402	0.5728	0.3816	1637.74	
2FI	7.29	0.7389	0.6184	0.3227	1793.90	
Quadratic	3.95	0.9411	0.8880	0.5398	1218.81	Suggested
Cubic	0.73	0.9988	0.9961	0.7306	713.51	Aliased

ANOVA (Table 8) suggested for the design is as follows. The Model F-value of 17.74 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case B, C, AB, AC, BC, A2 are significant model terms.

Table 8.ANOVA for Response Surface 2FI Model Analysis of variance table [Partial sum of squares - Type III] for fine particle fraction.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2492.47	9	276.94	17.74	< 0.0001	significant
A-Adition rate	*75.19*	*1*	*75.19*	*4.82*	*0.0529*	
B-Stabilizer conc.	*191.81*	*1*	*191.81*	*12.29*	*0.0057*	
C-Sonication Time	*1428.65*	*1*	*1428.65*	*91.53*	*< 0.0001*	
AB	*105.13*	*1*	*105.13*	*6.74*	*0.0267*	
AC	*78.13*	*1*	*78.13*	*5.01*	*0.0492*	
BC	*78.13*	*1*	*78.13*	*5.01*	*0.0492*	
A^2	*514.45*	*1*	*514.45*	*32.96*	*0.0002*	

B^2	*34.86*	*1*	*34.86*	*2.23*	*0.1659*	
C^2	*0.018*	*1*	*0.018*	*1.175E-003*	*0.9733*	
Residual	156.08	10	15.61			
Lack of Fit	*156.08*	*5*	*31.22*			
Pure Error	*0.000*	*5*	*0.000*			
Cor Total	2648.55	19				

Final Equation in Terms of Coded Factors:

Fine Particle Fraction (FPF) =

+66.97
+2.35 * A
+3.75 * B
+10.23 * C
-3.62 * A * B
+3.12 * A * C
+3.12 * B * C
-5.97 * A2
-1.56 * B2
+0.036 * C2……..(6)

Final Equation in Terms of Actual Factors:

Fine Particle Fraction (FPF) =

+66.96749
+2.34647 * Adition rate
+3.74768 * Stabilizer conc.
+10.22792 * Sonication Time
-3.62500 * Adition rate * Stabilizer conc.
+3.12500 * Adition rate * Sonication Time
+3.12500 * Stabilizer conc. * Sonication Time
-5.97474 * Adition rate2
-1.55532 * Stabilizer conc.2
+0.035667 * Sonication Time2……..(7)

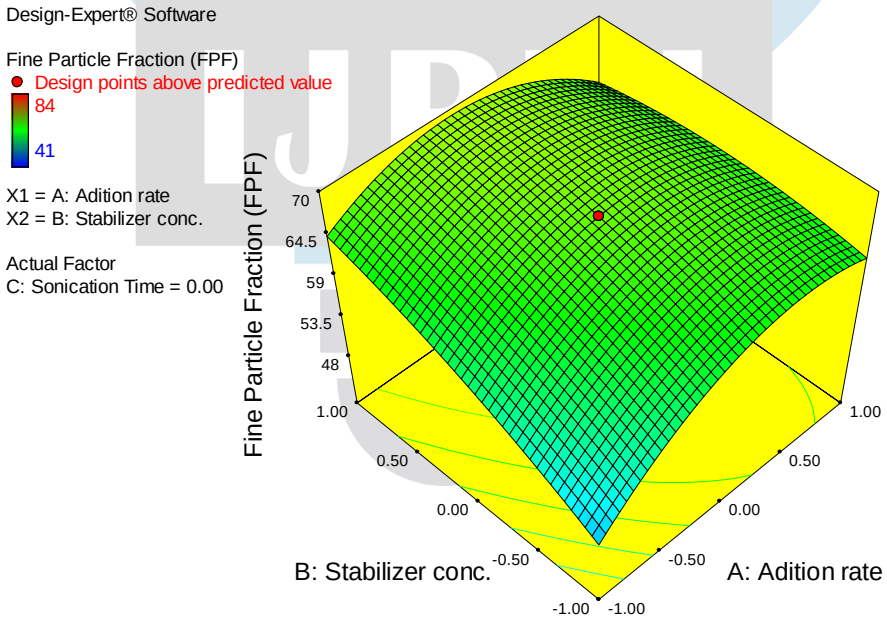


Figure 12. Response surface graph for fine particle fraction with respect to stabilizer conc. and adition rate as a prominent factors.

The fine particle fraction of BDP was shown in Figure 12. The fine particle fractions were the largest where there is higher amount of Adition rate, stabilizer concentration and sonication time. Statistical analysis of the particle size of microcrystal's produced by different formulations by a two-way ANOVA test showed that the concentration of the stabilizer and sonication time had a significant effect on the fine particle fraction of the microcrystal's while the adition rate did not have a significant effect on the fine particle fraction.

4.5.1.3 *Optimization of process parameters*

For optimization purpose we set constrains for the variable as follows**:**

Table 9.Constraints for the variables.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Adition rate	is in range	-1	1	1	1	3
Stabilizer conc.	is in range	-1	1	1	1	3
Sonication Time	is in range	-1	1	1	1	3
Mean Particle Size	is in range	1	5	1	1	3
Fine Particle Fraction (FPF)	maximize	41	84	1	1	3

Table 10.Formulations (up to 10) according to the descending order of desirability.

Number	Adition rate	Stabilizer conc.	Sonication time	Mean Particle Size	Fine Particle Fraction (FPF)	Desirability
F1	0.15	1.00	1.00	2.79784	82.6911	0.970
F2	0.13	1.00	1.00	2.80934	82.6874	0.969
F3	0.12	1.00	1.00	2.81383	82.6842	0.969
F4	0.19	1.00	1.00	2.77905	82.6825	0.969
F5	0.16	1.00	1.00	2.79669	82.6825	0.969
F6	0.07	1.00	1.00	2.83645	82.6524	0.969
F7	0.17	0.98	1.00	2.82671	82.6107	0.968
F8	0.28	1.00	1.00	2.73675	82.5846	0.967
F9	0.16	1.00	0.99	2.81129	82.5643	0.967
F10	0.20	0.93	1.00	2.88206	82.465	0.964

4.5.1.4 Evaluation of optimized formulation for their aerodynamic properties.

Optimised formulation F1 had evaluated by the deposition pattern by MSLI. The results indicated that the BDP particles of less fine fraction and spherical morphology had shown significantly better aerosol performance. Optimized product, corresponds to a decrease in stage 1 deposition along with a subsequently increase in stages 3 and 4 in the MSLI. It is worth that the particles were uniform in the presence of long time of sonication showed the highest FPF loaded and FPF emitted of 41 (2%) and 84(4%) respectively, depositing mainly on stages 3 and 4, with much lower amounts collected on the higher stages of the MSLI.

According to definition of D_{ae}

$$Dae = Deq\sqrt{\frac{\rho p}{\rho 0 x}} \qquad (8)$$

$$Deq = 3\sqrt{\frac{6V}{\pi}} \qquad (9)$$

Where *D*eq is the volume-equivalent diameter of the particle, *V* is the particle volume, ρ_p is the particle bulk density, ρ_0 is the unit density, and χ is the dynamic shape factor, defined as the ratio of the drag force on a particle to the drag force on the particle volume-equivalent sphere at the same velocity. Thus, *D*ae can be reduced by one or more of the following manipulations :(1) decreasing the volume-equivalent particle diameter(*D*eq), (2) reducing the particle density (ρ_p), and (3) increasing the particle dynamic shape factor (χ). Optimized budesonide particles had the thin thickness, and the spherical particles were calculated to be of higher *D*eq. The agglomerates formed by the spherical particles had a smooth surface, which effectively increased the interagglomerate distance and lowered the van der Waals attractive force. Furthermore, the surface asperities may also reduce the effective area of contact between agglomerates. Namely, the agglomerates had a lower bulk density. Third, the χ value for the particles of spherical shapes could be smaller. On account of the above reasons, the *D*ae of these spherical particles was intermediate so that they had high FPF values. [19]

4. Conclusion

In sonoprecipitation technique ultrasound accelerates the mass transfer when it propagates through a liquid medium, and initiates an important phenomenon known as cavitations during process. Cavitational bubbles are formed during the negative-pressure period of the sound wave which improves the micromeritic properties significantly and uniformly. When a cavitational bubble implodes, a localized hot spot with a high temperature and pressure is formed releasing a powerful shock wave which proves this technique is lesser significance in production of heat sensitive drug. The reason for the above phenomena is that the collapse process is very rapid and significantly uniform. Application of ultrasound is uniform throughout the process and it is observed that there was highest fine particle fraction as compared to other previous techniques.

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