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Formulation and Evaluation of Microparticles Formed by In Situ Micronization Technique: Optimization of Process Parameters

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ABSTRACT

Aceclofenac is a non steroidal anti- inflammatory drug characterized by low solubility and high permeability which corresponds to BCS class II drug. The strategy of increasing the *in vitro* dissolution has the potential to enhance the oral bioavailability when using nanosized crystalline drugs. The purpose of this study was to evaluate a novel *in situ* micronization method avoiding any milling techniques to produce nano- or microsized drug particles by controlled crystallization to enhance the dissolution rate of poorly water-soluble drugs. Aceclofenac microcrystals were prepared by the association of the previously molecularly dispersed drug using a rapid solvent change process. The drug was precipitated in the presence of stabilizing agents, such as hydrocolloids. The obtained dispersion was spray-dried. Particle size, morphology, flow property, zeta potential and dissolution rate were analyzed. Physicochemical properties were characterized using differential scanning calorimetry and X-ray diffractometry. The obtained dispersions showed a homogeneous particle size distribution. Drugs are obtained in a mean particle size of approximately 3µm and below. A high specific surface area was created and *in situ* stabilized. The surface was hydrophilized because of the adsorbed stabilizer. The solubility of the drug was increased by 2 folds. Thus, a drug powder with markedly enhanced dissolution rate was obtained. *In situ* micronization is a suitable method for the production of micro-sized drugs. This technique can be performed continuously or discontinuously and uses only common technical equipment. Compared to milled products drug properties are optimized as all particle surfaces are naturally grown, the particle size is more uniformly distributed and the powder is less cohesive.

Keywords: Aceclofenac, microparticles, in situ micronization, particle size, solubility, dissolution rate.

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INTRODUCTION

Many drugs are poorly soluble or insoluble in water, which results in poor bioavailability because the solubility of a drug is an important factor in determining the rate and extent of its absorption[1] For class II-drugs, according to the biopharmaceutics classification system[2], the dissolution rate is the limiting factor for the drug absorption rate. Also for class IV-drugs the dissolution rate can be the limiting factor. An enhancement in the dissolution rate of these drugs can increase the blood-levels to a clinically suitable level[3]. One way to improve the dissolution rate is to reduce particle size, which increases the total surface area[4] .

Several methods of reducing particle size have been suggested. Physical methods such as milling and grinding [6] are successful in particle size reduction; however the particle size uniformity is not achieved. A common particle size reduction method for hydrophobic drugs is microcrystallization[6] A common method for increasing the dissolution rate is to form a high specific surface area by micronization. The process which is usually used to obtain small particles is the disruption of large crystals. Chaumeil[7] describes the improvement in dissolution rate and in bioavailability by micronization of sparingly water-soluble drugs using jar mills and fluid energy mills.

However, these methods have several disadvantages resulting from the mechanical disruption process. The micronization process using mills is extremely inefficient[8] because of the high-energy input that can alter the surface properties as a thermodynamically activated surface is created[9,10]. Even a small amount of activated material at the surface affects the drug substance properties, such as the blending characteristics[11] or flow properties[12] When the partially amorphous surface recrystallizes, the physical properties of the drug change. The conversion of crystalline solid surfaces into partially amorphous solid surfaces leads to a “dynamic nature” of the micronized drug[13]. The newly created surfaces are not naturally grown because the cleavage plane is the crystal face with the smallest attachment energy[14.] Thus, this surface will dominate the size-reduced particles and the milled powder is characterized by their surface properties[15]. The high pressures used in homogenization process cause changes in the crystal structure, and as a result, the amorphous fraction in the particle increases[16]. Thus milling affects several physical properties of the drug, such as powder flow, agglomeration behavior, or electrostatic behavior. Beside these effects, the chemical reactivity or degradation also can be affected by milling [17,18.] Even the particle size can change during storage after micronization because of stress relaxation processes[19]. Beside these problematic properties, a further disadvantage especially of jet-milling

processes, is a broad size distribution [20]. Because of abrasion, the product can be afflicted with metallic impurities that can affect the chemical stability as a result of catalytic activity.

However, the preparation and stabilization of small particles are not easy because of their tendency to grow. Because of the high structure that must be created, the established methods need high amounts of drugs are prepared by a solvent change process that precipitates and stabilizes the drug in a small particle size by the use of hydroxypropylmethylcellulose (HPMC)[21] . As HPMC shows surface activity [22] it can be adsorbed onto the newly created surface of the precipitated drug in order to lower the interfacial tension. After drying this dispersion, a drug powder with a high drug load is obtained[21]. As the drug powders are prepared directly in micronized state during particle formation without any size reduction, this technique can be described as an in situ micronization technique [23]

In situ micronization is a suitable method for the production of micron-sized drugs. This technique can be performed continuously or discontinuously and uses only common technical equipment. Compared to milled products drug properties are optimized as all particle surfaces are naturally grown, the particle size is more uniformly distributed and the powder is less cohesive [15].

Aceclofenac (2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid) is COX2 inhibitor, widely used for the treatment of ankylosing spondylitis, rheumatoid arthritis and osteoarthritis. It has a low solubility in water (0.18 mg/ml) and gastric fluids, [24] which determines a low dissolution rate and hence interindividual variability on its bioavailability. To enhance the dissolution rate of this drug, solid dispersion with peg6000 was prepared which increased the dissolution by 2-3 times [25]. In another study, aceclofenac was granulated with neusilin 2 which increased its dissolution rate by twice the original [26]

In order to avoid thermodynamically activated sites in size reduction techniques by milling, micron-sized particles are prepared by solvent change method [23] to enhance the dissolution and consequently the absorption of drugs.

As discussed by Rasenak and Muller[15], in situ micronization by the presence of a stabilizing polymer covers the hydrophobic surfaces of the precipitated substances and consequently the steric hindrance caused by the polymer prevents the crystal growth. In this study an in situ micronization technique based on solvent change method was used for size reduction and hence dissolution and bioavailability enhancement of aceclofenac.

MATERIALS AND METHODS

Materials

Aceclofenac was used as a model drug and was a generous gift from Aarti Drug Distributors, Mumbai (India). HPMCE5LV which was used as a stabilizing agent was obtained from Loba Chemie Pvt. Ltd, Mumbai. Acetone of chromatographic grade was purchased from Finar Chemicals Ltd. All other chemicals and solvents were of reagent grade.

Methods

Preparation of microparticles

The in situ micronization process was carried out using solvent change method by instantaneously mixing two liquids in the presence of HPMCE5 LV as stabilizing agents. The process was carried out at room temperature. In the first step, aceclofenac (0.5-1 g) was dissolved in 20 ml of acetone (as the solvent) and 0.01 or 0.1 g of stabilizing agent in 100 ml of water (as non-solvent). By batch-wise mixing of the non-solvent that was poured rapidly into the drug solution under stirring at 30,000 rpm using an ultra-homogenizer (Heidolph, Silent crusher M, Germany), a micronfine dispersion was formed spontaneously. The mixture was allowed to be mixed for 15 min and then it was spray dried. The parameters, inlet temp (80^0C), outlet temp (60^0C), aspirator flow rate (45 Nm3/hr) and feed pump flow rate (3 ml/min) were set for spray drying.

Experimental Design

Experimental runs (Batches) for optimization using Central composite design (CCD) are designed by considering two independent variables:

I) X_1 = Drug Conc.

II) X_2= Stabilizer Conc.

Details of process are given in table 1 and the response values for design are mentioned in table 2 and the actual batches obtained from the design are mentioned in table 3.

Table 1 Summary for designing of batches

Factor	Name	Units	Type	Subtype	Min	Max	-1	+1	Mean	SD
A	Drug Con.	gm/20ml	Numeric	Continuous	0.5	1	0.57	0.93	0.75	0.14
B	Stabiliser Con.	% w/v	Numeric	Continuous	0.02	0.1	0.03	0.09	0.06	0.02

Table 2 Responses Selected for Optimization

Response	Name	Unit	Obs	Analysis
Y1	Particle size	microns	13	Polynomial
Y2	Solubility	mg/ml	13	Polynomial
Y3	Drug Content	%	13	Polynomial
Y4	In vitro drug release	%	13	Polynomial

Table 3 Experimental batches designed by using central composite design (CCD)

Batch No.	STDS	Runs	Drug Conc.	Stabilizer Conc.
I1	11	1	0.75	0.06
I2	12	2	0.75	0.06
I3	4	3	0.93	0.09
I4	3	4	0.57	0.09
I5	9	5	0.75	0.06
I6	10	6	0.75	0.06
I7	1	7	0.57	0.03
I8	6	8	1.00	0.06
I9	2	9	0.93	0.03
I10	5	10	0.5	0.06
I11	13	11	0.75	0.06
I12	8	12	0.75	0.10
I13	7	13	0.75	0.02

Evaluation :

Percentage yield:

The percentage yield of the experiment was determined by using the formula given as:

Process efficiency = Practical yield of drug (gm)/ Wt. of drug taken (gm) × 100

Drug content

Drug content was analyzed by taking 10 mg of Aceclofenac drug sample and dissolved in 100 ml of Phosphate Buffer pH 7.5. Each of these solutions was further diluted with phosphate buffer pH 7.5. Absorbance was measured on UV-visible spectrophotometer at 275nm. Drug content was determined by using the formula

$$\text{Percent drug content} = \frac{\text{Test abs.} \times \text{Standard conc.}}{\text{Standard abs.} \times \text{wt. of drug}} \times \text{Dilution factor} \times 100$$

Particle size: [12,13]

The average particle size of aceclofenac microparticles were measured by the method of laser light diffraction using Malvern Mastersizer Micro Ver. 2.19 (Malvern Instruments Ltd, UK). Prior to measurements, about 50 mg of each sample were dispersed with 100 ml of hexane. The particle size distributions were estimated by setting the intensity of the scattered light at wavelength of 750 nm and the scattering angle (θ) of 90^0.

Saturation solubility: [4,5,6]

Saturation Solubility of aceclofenac microparticles was determined in distilled water. Excess of drug 200 mg was added in each cap vial containing 5 ml Distilled Water. Each vial was in sonicated for 15 min so that excess amount of drug gets dissolved up to super saturation and

some drug is kept in suspended form. The vials were kept in orbital shaker for 48 hrs for stirring. The selected quantity of sample was centrifuged at 7500 rpm for 15 min so that excess amount of supernatant obtained. The drug in supernatant was analyzed by making proper dilution with selected ratio of solvent by UV-Spectrophotometer at 272 nm to calculate the solubility of drug.

Fourier transform infrared spectroscopy

Infrared spectrum of Aceclofenac and its microparticles was determined on Fourier Transform Infrared Spectrophotometer (FT/IR 4100, Jasco) using KBr dispersion method. The base line correction was done using dried potassium bromide. The samples to be analysed and KBr were previously dried in oven for 30 min and mixed thoroughly with potassium bromide in 1:25 (sample: KBr) ratio in a glass mortar. These samples were then placed in a sample holder and scans were obtained at a resolution of 2 cm-1 from 4000 to 400 cm-1**.**

Zeta Potential

Formulation (0.5 mL) was diluted to 50ml with distilled water in glass beaker with constant stirring. Zeta-potential of the resulting suspension was determined using the Zetasizer (model: Nano ZS, Malvern Instruments, Westborough, MA, USA) Electrophoretic mobility (μm/s) was measured using small volume disposable zeta cell and converted to zeta potential by in-built software using Helmholtz–Smoluchowski equation. All determinations were made in triplicate.

Flow properties of product: [18]

The obtained powder sample by SAA processing was further evaluated for its flow properties like angle of repose, bulk density, tapped density, hausner's ratio, compressibility index. These were the important properties which were needed to be studied before formulation into dosage form. Reports of these studies were given in results.

Polydispersity index

The PDI determination was using photon correlation spectroscopy with in-built Zetasizer (model: Nano ZS, Malvern Instruments, Westborough, MA, USA) at 633 nm.

Differential Scanning Calorimetry (DSC):[19,20]

DSC measurements were performed on a differential scanning calorimeter equipped with an intra-cooler (DSC Mettler STAR SW 9.20, Switzerland). Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min. All accurately weighed samples (about 5-10 mg of samples) were placed in a sealed aluminum pan, and the samples were heated under nitrogen gas flow (20 ml/min) at a scanning rate of 10 ^{0}C per min from 40 to 300^{0}C. An empty aluminum pan was used as reference.

Powder X-Ray Diffraction Study (XRD): [6,21]

X-ray diffraction patterns of the powdered samples of the drug and carrier were recorded using Philips PW3710 Analytical XRD B. V. X-ray diffractometer using Cu K 2α rays with a voltage of 40 kV and a current of 25 mA. Samples were scanned for 2θ from 5 to 80^0. Diffraction pattern for aceclofenac microparticles was obtained.

Scanning Electron Microscopy (SEM): [12,14]

Morphological characteristics of sonoprecipitated aceclofenac powder was analyzed by a Scanning Electron Microscope (JEOL, JSM-6360A, Japan). Powder was dispersed on a carbon tab previously stuck to an aluminum stub. Samples were coated with gold-palladium (layer thickness 250Å) using a sputter coater.

Optical microscopy

Optical microscopy of the drug sample was carried out by using, Motic Digital Microscope. Very slight quantity of the powder sample was spread on the glass slide by using fine haired brush. This slide was focused under various magnification lenses and the pictures were captured.

In vitro drug release study:[22]

In vitro drug release study was carried out according to the guidelines given in USP 30- NF24. Test was performed by using USP apparatus II (Basket type). The volume of medium used was 900ml of Distilled water. The temperature was maintained at 37± 2^0c. The RPM of the study was kept at 50. 5ml aliquots were withdrawn at time interval of 10 min and the study was carried for duration at which drug shows almost complete release from the capsule. According to the USP standards at least not less than 70% (Q) of the labeled amount of $C_{16}H_{13}Cl_2NO_4$ should get released (USP30).

RESULTS AND DISCUSSION

Evaluation of Batches

Percentage Yield

The percent yield was calculated from theoretical & practical yield. Since spray drying was employed to obtain the microparticles in dried form, the percent yield was affected. It was found to be in the range of 65-70%. The results of percent yield are given in table 4 & 5.

Table 4 Percent yield of batches I1-I7

Batch	I1	I2	I3	I4	I5	I6	I7
Percentage Yield	63.24	65.12	70.31	69.54	62.31	59.36	67.29

Table 5 Percent yield of batches I8-S13

Batch	I8	I9	I10	I11	I12	I13
Percent Yield	68.34	61.37	64.21	61.78	72.54	56.34

Drug Content

Drug content in the microparticle batches (I1-I13) are analysed by UV spectrophotometer at 272 nm. The results of drug content are given in table 6 and 7.

Table 6 Drug content of batches I1-I7

Batch	I1	I2	I3	I4	I5	I6	I7
Drug Content (%)	75.39	82.34	86.34	79.24	76.15	77.56	81.2

Table 7 Drug content of batches I8-S13

Batch	I8	I9	I10	I11	I12	I13
Drug Content (%)	80.64	85.64	72.64	76.41	73.54	79.12

Drug content in batch I3 and I9 was found to be highest. Batch I10 and I12 showed lowest % drug content in microparticles. This may be due to the drug and stabilizer concentration. Thus higher the stabilizer and drug concentration, it shows highest encapsulation of drug and hence the drug content was found to be maximum for I3 and I9 batches.

Particle Size

Particle Size Determination

Table 8 Average Particle size of batches I1-I7

Batch	I1	I2	I3	I4	I5	I6	I7
Particle Size (microns)	3.45	3.64	4.06	3.67	3.78	3.59	3.86

Table 9 Average Particle size of batches I8-I13

Batch	I8	I9	I10	I11	I12	I13
Particle Size (microns)	3.97	4.35	3.12	3.82	2.98	5.36

The average particle diameter of the aceclofenac after complete processing of the drug is shown in table 8 and 9. The average particle size of aceclofenac when processed by in situ micronization technique is considerably smaller than the unprocessed aceclofenac. The unprocessed aceclofenac shows particle diameter average upto 200 μm while after processing it shows particle diameter ranging from 3.5-5 μm which is suitable for the many of the formulations like powder for inhalation. This may be due to the fact that as the drug concentration increases, the supersaturation and nucleation rate of drug is enlarged and the particle size became smaller. High drug loading can generate higher supersaturation which favors more formation of nuclei, but which may cause the collision, agglomeration and growth of numerous nuclei within certain region. At the lowest stabilizer concentration, the average particle size was around 5 μm, which can be explained as the coverage of stabilizer on the microcrystal surface was inadequate to arrest particle growth. The particle size reduced with the increasing of stabilizer concentration. [14]

Particle Size Distribution

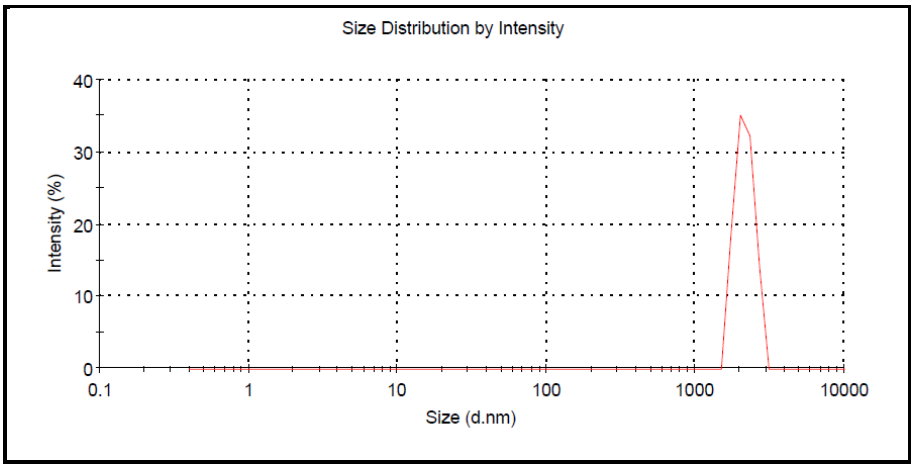

Figure 1. Particle Size Distribution Of Batch I3

The figure 1 represents particle size distribution of in situ micronized drug. The mean diameter was found to be 3.401 micrometer. The particle size distribution was found to be narrow which is indicated by the sharp peak. Thus all the particles were found to be uniform in size. The reason behind uniform particle size distribution may be due to increased supersaturation which leads to increased nucleation and inhibition of crystal growth.

Saturation Solubility

Saturation Solubility of the microparticle batches (I1-I13) are analysed by UV spectrophotometer at 272 nm. The results of saturation solubility are given in table 10-11.

Table 10 Saturation Solubility of batches I1-I7

Batch	I1	I2	I3	I4	I5	I6	I7
Solubility (mg/ml)	0.263	0.256	0.278	0.232	0.243	0.267	0.286

Table 11 Saturation Solubility of batches I8-S13

Batch	I8	I9	I10	I11	I12	I13
Solubility (mg/ml)	0.246	0.301	0.291	0.235	0.258	0.192

The values of solubility showed that there was significant change in solubility of aceclofenac after processing by in situ micronization technique. Initially unprocessed aceclofenac showed saturation solubility of 0.15 mg/ml in distilled water and later it was increased significantly ranging between 0.19 to 0.286 mg/ml as illustrated in figure. 3. Thus the results indicate that the solubility has increased twice than that of original. I9 and I10 showed the maximum solubility in water while batches I13 showed the minimum solubility amongst all other batches. This may be due to a, particle size of the drug obtained was smaller, which exposes higher surface area to solvent which increase the solubility of the Drug in Water[1] and like ethanol, acetone also has good diffusivity property which generates the porous powder, which has good water uptake capacity and hence the solubility of the powder obtained was higher[27].

FTIR

Microparticles produced by in situ micronization has been studied by FT-IR spectrum. It has been observed that all the characteristic peaks were retained but the intensity has been reduced in graphs of FTIR spectra, confirming compatibility of Aceclofenac with all excipients. These results indicate that there is no chemical or structural change in drug (Figure 6).

Table 12 IR interpretation data

Sr. No.	Remarks	Peak cm^{-1} (Observed)
1.	N-H Stretching	3327.57
2.	O-H Stretching	3025.76, 2094.32
3.	C-O stretching	1707.66
4.	skeleton vibration of aromatic C-C stretching for NH	1513.85
5.	O-H in plane bending	1339.32
6.	CN aromatic amine	1250.61
7.	O-H out plane bending	908.308

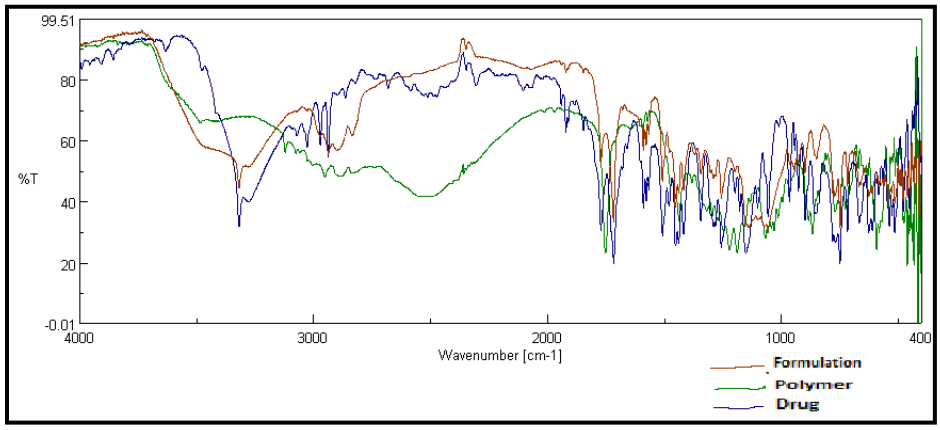

Figure 2. IR Spectra Of Drug, Polymer And Formulation

Zeta Potential Determination:

The figure 3 represents the zeta potential of insitu micronized drug. The average zeta potential was found to be -21.5mV. This value indicates that the formulation has medium stability.

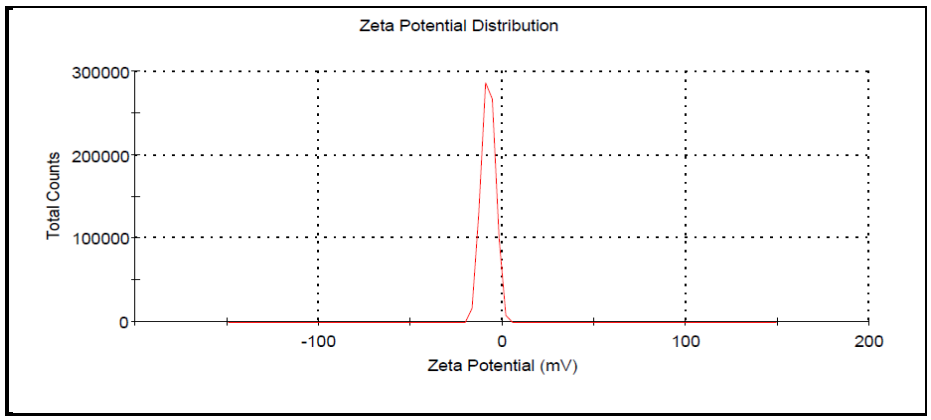

Figure 3. Zeta Potential Distribution Curve of Formulation (I3)

Flow Properties:

Table 13 Flow properties of Batches I1-I13

Optimized Batch No.	Angle of repose (^{0})	Bulk density (gm/cm^3)	Tapped Density (gm/cm^3)	Carr's index	Hausner's ratio
I1	30.38±0.497	0.343±0.001	0.434±0.001	20.87±0.427	1.26±0.01
I2	31.65±0.175	0.325±0.001	0.420±0.001	22.38±0.373	1.256±0.049
I3	28.51±0.396	0.367±0.002	0.461±0.001	20.34±0.384	1.25±0.01
I4	29.67±0.265	0.359±0.002	0.448±0.002	19.80±0.742	1.246±0.011
I5	31.91±0.256	0.373±0.001	0.458±0.001	18.48±0.370	1.223±0.005
I6	31.03±0.462	0.351±0.0005	0.439±0.002	19.95±0.545	1.243±0.045
I7	30.06±0.370	0.365±0.001	0.467±0.001	21.77±0.346	1.275±0.005
I8	29.66±0.560	0.340±0.001	0.433±0.002	21.40±0.369	1.267±0.005
I9	33.34±0.386	0.367±0.002	0.461±0.001	20.32±0.664	1.253±0.011
I10	32.74±0.639	0.348±0.002	0.436±0.001	20.23±0.291	1.246±0.005
I11	31.55±0.275	0.336±0.002	0.418±0.002	19.59±0.100	1.244±0.008
I12	28.61±0.480	0.370±0.001	0.474±0.001	21.86±0.431	1.286±0.028
I13	29.87±0.276	0.340±0.002	0.425±0.003	19.84±0.886	1.243±0.015

*flow property represent mean ± SD, n = 3 determinations

Microparticles of the aceclofenac were evaluated for its flow properties like Bulk density, Tapped density, Angle of repose, Compressibility properties and Hausner's ratio[26] The results of this analysis were shown in table 13.These reports shows that the formed particles have the flow properties within the passable range, and which is best suitable in both tablet as well as powder for inhalation formulations. This may be probably due to formation of spherical particles by in situ micronization technique, since the powder shows uniform distribution, the flow properties of drug are modified.[15]

Polydispersity Index

The polydispersity index was found to be 0.271 which was found to be less than 1. Thus it indicates that the suspension is monodispersed.

Differential scanning calorimetry

The DSC scan of unprocessed aceclofenac showed a single endotherm at 152^{0}C ascribed to melting of drug (Figure. 4). However, spray dried sonicated aceclofenac did not show melting endotherm at 152^{0}C and exhibited endothermic change in the heat capacity at 125^{0}C. It proved that the sonicated aceclofenac was in partially crystalline and partially amorphous form. The endothermic curve was decreased in size indicated that amorphous character has been introduced in the microparticles.

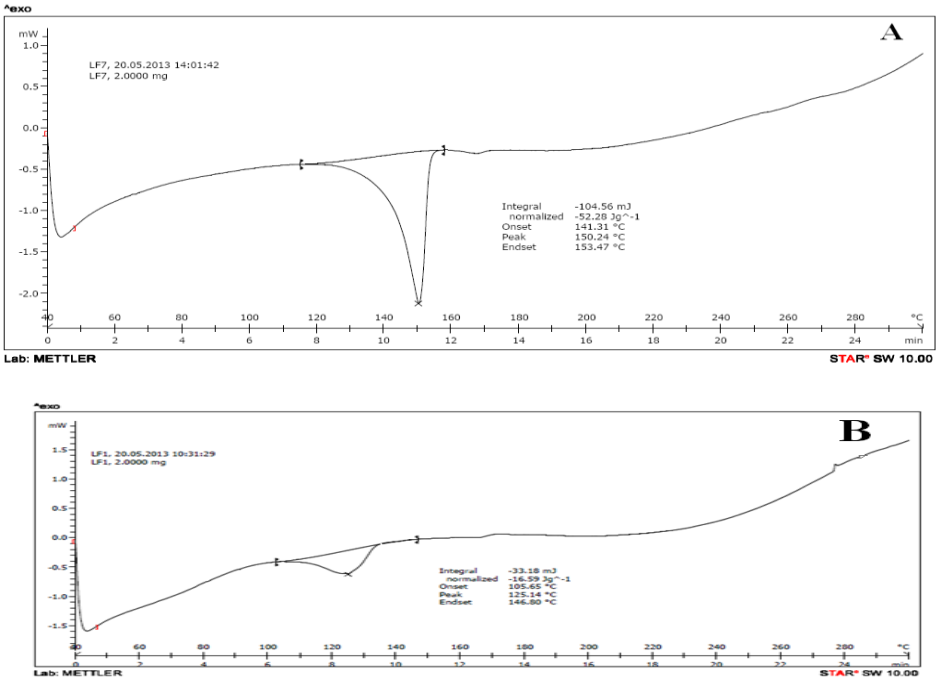

Figure 4 DSC Curves Of A) Drug B) Formulation

Powder X-ray diffraction study

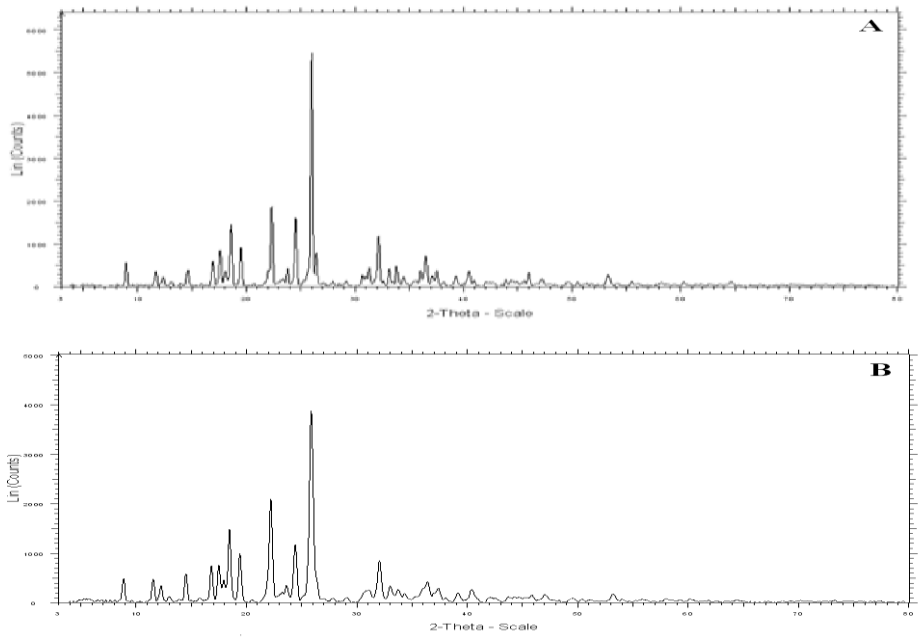

Figure 5. XRD Graph Of A) Drug B) Formulation

X-ray diffraction patterns of figure. 5 revealed that pure drugs were in crystalline state as it showed sharp distinct peaks notably at 2θ diffraction angles of 17.53, 18.53, 19.44, 22.27, 24.5, 25. 94, 32.210 with 838,1450,904,1868,1604,5471,1175 intensities for Aceclofenac respectively. The reflections (specific peaks) corresponding to the drug were found in the formulation diffractogram with reduced intensity as 751,1424,889,1094,1164,3880,838 respectively as

compared to drug alone. The reduction in intensity and number of typical diffraction peaks in formulation diffractogram suggests reduction in crystalline nature of drug and may be converted from crystalline to amorphous form.

Scanning Electron Microscopy (SEM)

Figure 6 A and 6 B represents the morphology of unproceesed drug and in situ micronized drug respectively. When aceclofenac is spray dried out of solution, resulting powders have a characteristic morphology. The drying starts at the outside of the atomized droplets. Solvent evaporates and the concentration of dissolved substance increases until the solubility limit is reached. A solid shell forms on which further dried substance can attach. Some rudimental hollow spheres can be observed, but the majority of the particle collective has a different shape. In this case the particle development is completely different due to the fact that the primary particles are already formed during the precipitation.

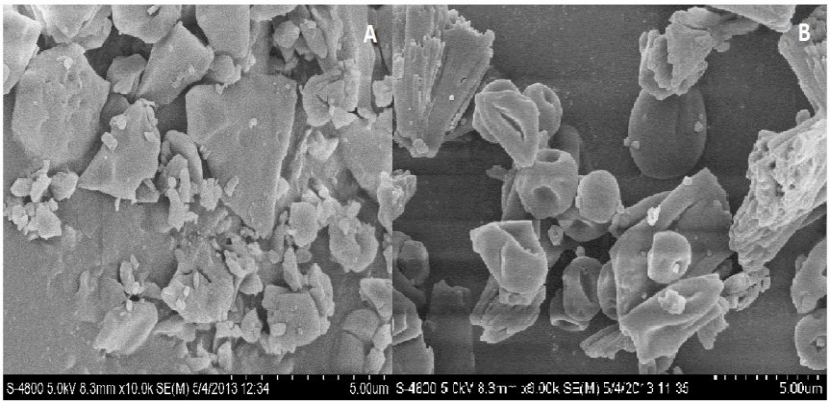

Figure 6 SEM Images Of A) Durg B) Formulation

Optical microscopy

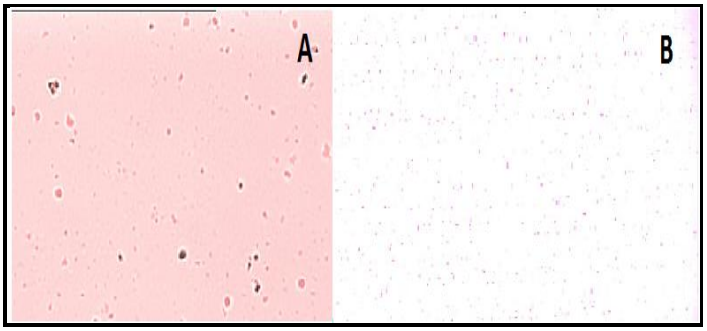

Figure 7 Optical Microscopic Image Of A) Drug B) Formulation

Figure. 7 shows the optical microscopic images of the Aceclofenac after processing by in situ micronization. The image shows a very sharp distribution of drug particles after processing; there appears some clusters of the particles due to generation of static charges arises due to micronization. All I1-I13 batches shows very fine and narrow distribution of the particles.

In Vitro Drug release profile

Table 14 Dissolution Profile of batches I1-I5

Time(min)	I1	I2	I3	I4	I5
0	0.000	0.000	0.000	0.000	0.000
5	10.23	11.22	13.69	12.63	13.85
10	37.36	38.54	33.79	35.29	34.44
15	49.82	46.55	49.67	47.47	43.75
20	58.55	56.72	60.82	55.98	56.34
25	66.33	64.13	66.42	63.44	62.83
30	73.45	75.28	73.26	70.99	71.11
45	79.29	80.19	82.16	78.36	78.98
60	85.34	83.73	88.33	80.80	84.62

Table 15 Dissolution Profile of batches I1-I10

Time(min)	I6	I7	I8	I9	I10
0	0.000	0.000	0.000	0.000	0.000
5	13.05	11.88	12.36	11.64	13.24
10	37.18	35.69	36.28	32.12	33.64
15	48.26	52.67	50.37	51.47	46.18
20	60.38	61.78	60.84	59.55	62.15
25	68.03	69.15	67.28	66.25	70.16
30	76.37	73.28	74.17	70.64	78.24
45	80.16	80.46	78.38	77.89	83.67
60	85.23	86.29	80.36	79.63	87.28

Table 16 Dissolution Profile of batches I11-I13

Time(min)	I11	I12	I13
0	0.000	0.000	0.000
5	10.63	11.66	12.36
10	34.85	31.29	37.19
15	52.48	49.35	43.96
20	61.39	59.37	51.68
25	69.54	66.24	60.87
30	73.41	69.68	65.22
45	80.16	76.36	75.41
60	88.37	79.27	78.23

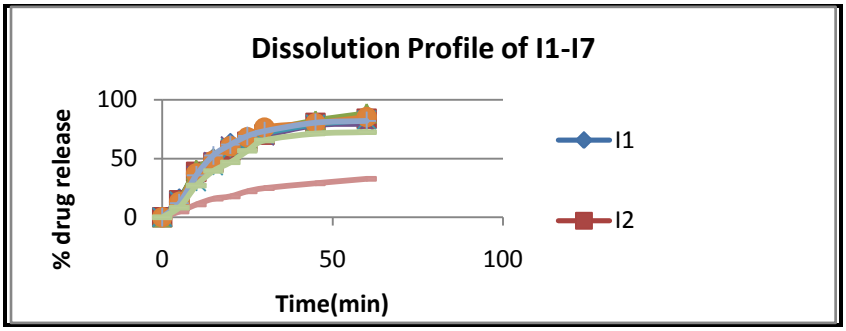

Figure 8. Dissolution Profile of Batches I1-I7 Along With Unprocessed Drug and Marketed Formulation

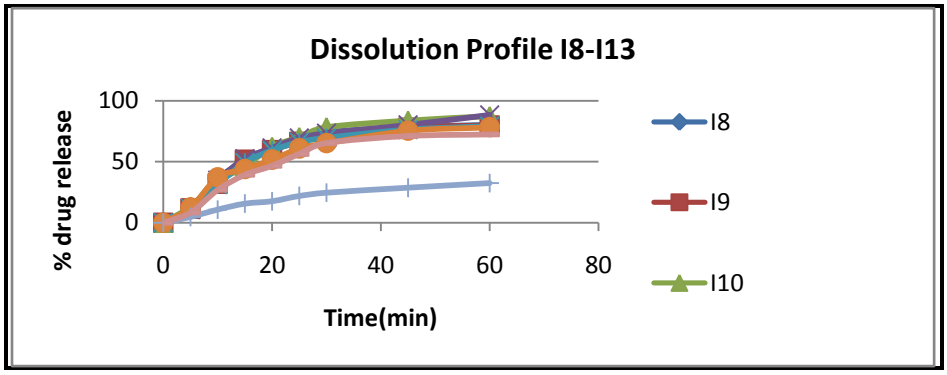

Figure 9. Dissolution Profile of Batches I8-I3 Along With Unprocessed Drug and Marketed Formulation

The *in vitro* dissolution profile of the drug (Aceclofenac) was carried as per the USP I (Basket type) apparatus (Electrolab-TDL-08L) specifications and the results of the *in vitro* dissolution study are shown in table 14-16. All the batches I1-I13 shows the better release profile than the pure unprocessed drug. The batches I1-I13 shows upto 75% of drug release within 30 min of study. Batches I2, I3 and I10 showed the rapid release of drug within 30 min of study. Batches I6 and I10 showed the maximum release. All the batches I1-I13 shows the release kinetics by first order drug release. It showed the r^2 value upto 0.9869 and 'K' value was within 12.5043**.** These results pass the IP specifications for the aceclofenac [26.] These batches show the rapid release of drug from its formulations. The Batches with greater solubility shows the rapid dissolution of drug. As the particle size of the in situ micronized aceclofenac is lesser, drug shows higher solubility which results into rapid release of the drug. Aceclofenac shows complete release of the drug from formulation but its onset of action is late. After processing, onset was found to be quicker which may reduce dose requirement [28]. Graphs of the drug release from capsules were given in the Figure. 8-9. which proves the release rate and its kinetics of drug release. This rapid release may be due to micronization of drug particles and increased solubility of drug, which ultimately enhances the dissolution of drug. Solubility depends on particle size, generated porosity and increased water uptake capacity of drug, which rapidly dissolves drug in medium.

Analysis of Data using Design Expert:

Table 17 Regression Results of measured responses

Responses	Standard Deviation	R Squared	Adjusted R Squared	Adequate Precision	Model
Particle Size	0.15	0.9677	0.9354	21.639	Cubic
Solubility	0.013	0.9286	0.7848	9.705	Quartic
Drug Content	1.24	0.9598	0.8794	13.313	Quartic
Drug Release	2.36	0.7467	0.5658	5.810	Quadratic

The "Pred R-Squared" must be equal or nearer to "Adj R-Squared". "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here we can see that R squared values for all the responses are in reasonable agreement with Adjusted R- Squared value & the ratio of "Adeq Precision" is greater than 4 in all cases. Thus the respective models selected for the respective respone can be used to navigate the design space.

Effect on Particle Size:

ANOVA for Response Surface Reduced Cubic Model

Table 18 ANOVA results for Response Surface Reduced Cubic Model

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	4.04	6	0.67	29.28	0.0003 Significant
A- Drug Conc.	0.54	1	0.54	24.12	0.0027
B- Stabilizer Conc.	2.83	1	2.83	126.06	< 0.0001
AB	2.500E-003	1	2.500E-003	0.11	0.7500
A^2	3.883E-003	1	3.883E-003	0.17	0.6921
B^2	0.58	1	0.58	25.84	0.0023
A^2B	1.04	1	1.04	46.34	0.0005
Lack of fit	0.045	2	0.023	1.02	0.4391 Not significant

The Model F-value of 29.98 implies the model is significant. There is only a 0.03% 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 A, B, B^2, A^2B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 1.02 implies the Lack of Fit is not significant relative to the pure error. There is a 43.91% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

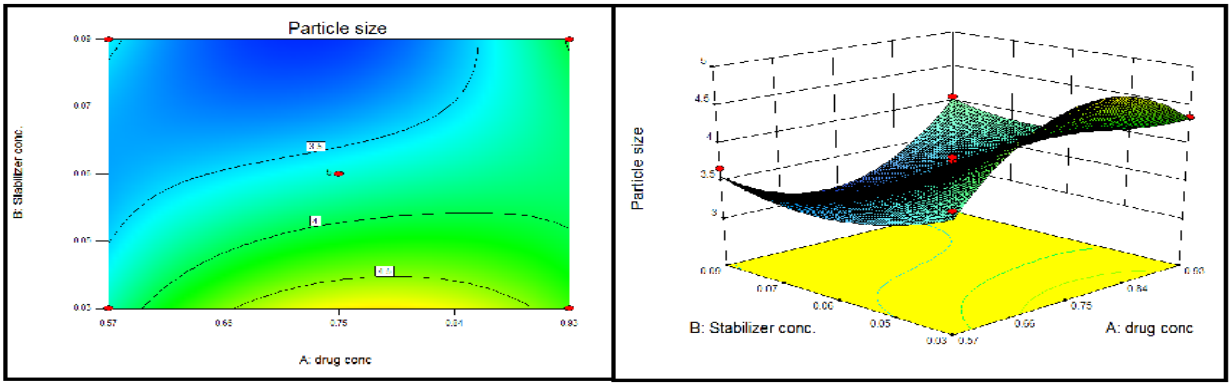

Figure 10 Effect of Stabilizer conc. and drug conc. on Particle Size

Final Equation in Terms of Coded Factors:

Particle size = + 3.66 + 0.26 * A - 0.84 * B - 0.025 * A * B - 0.024 * A^2 + 0.29 * B^2 + 0.72 * A^2 * B

Final Equation in Terms of Actual Factors:

Particle size = - 22.56145 + 76.36745 * drug conc + 389.80122 * Stabilizer conc. - 1229.35325 * drug conc * Stabilizer conc.- 49.73013 * drug conc ^{2} + 361.09375 * Stabilizer conc.^{2} + 816.23550 * drug conc ^{2} * Stabilizer conc.

The low to high-drug loading were set in between 0.5 to 1 gm/ 20 ml. It can be seen that with an increase in drug loading, the particle size increased(Figure 10). High drug loading can generate higher supersaturation which favors more formation of nuclei, but which may cause the collision, agglomeration and growth of numerous nuclei within certain region. Taken all aspects into account, drug loading was chosen as 0.57 gm/20 ml.

The effect of the concentration of stabilizer on the particle size was tested, and the result was as Figure 10. At the lowest stabilizer concentration, the average particle size was around 3 μm, which can be explained as the coverage of stabilizer on the microcrystal surface was inadequate to arrest particle growth. The particle size reduced with the increasing stabilizer concentration. The optimum stabilizer concentration selected was found to be 0.05% w/v.

Effect on Solubility

ANOVA for Response Surface Quartic Model (Alliased)

Table 19 ANOVA for Response Surface Quartic Model (Alliased)

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	9.430E-003	8	1.179E-003	6.47	0.0445 significant
A-Drug Conc.	1.012E-003	1	1.012E-003	5.56	0.0779
B- Stabilizer Conc.	2.178E-003	1	2.178E-003	11.95	0.0259
AB	2.402E-004	1	2.402E-004	1.32	0.3148
A^2	3.521E-004	1	3.521E-004	1.93	0.2368
B^2	1.104E-003	1	1.104E-003	6.06	0.0696
A^2B	3.627E-003	1	3.627E-003	19.91	0.0111
AB^2	1.942E-003	1	1.942E-003	10.66	0.0309
A^2B^2	1.513E-003	1	1.513E-003	8.30	0.0450

The Model F-value of 6.47 implies the model is significant. There is only a 4.45% 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, A^2B, AB^2, A^2B^2 are significant model

terms. Values greater than 0.1000 indicate the model terms are not significant.

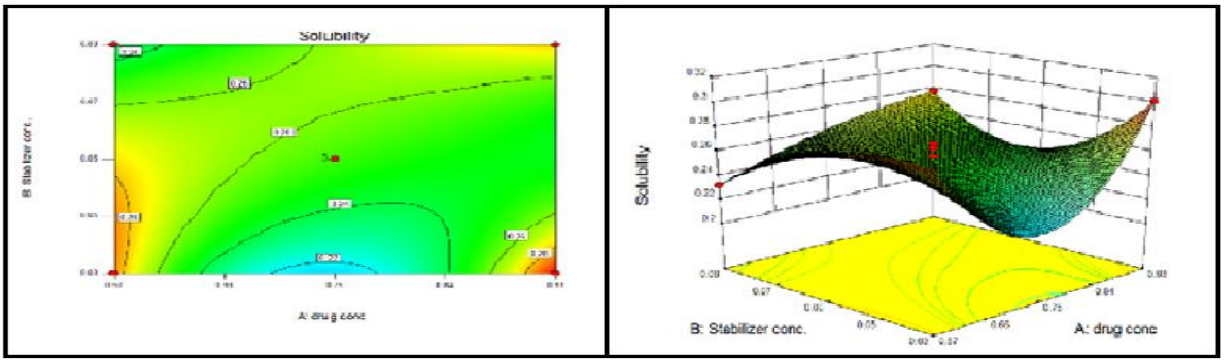

Figure 11 Effect of Stabilizer conc. and drug conc. on Solubility

Final Equation in Terms of Coded Factors:

Solubility = +0.25 -0.016 * A +0.023 * B +7.750E-003 * A * B +7.850E-003 *

A^2 -0.014 * B^2 -0.043 * A^2 * B +0.031 * A * B^2 +0.027 * A^2 * B^2

The low to high-drug loading were set in between 0.5 to 1 gm/ 20 ml. It can be seen that with an increase in drug loading, the particle size increased(Figure 11). High drug loading can generate higher supersaturation which favors more formation of nuclei, but which may cause the collision, agglomeration and growth of numerous nuclei within certain region. The particle size has direct impact on solubility according to Noyes Whitney equation. Thus the solubility was found to be increased with decrease in particle size. Taken all aspects into account, drug loading was chosen as 0.57 gm/20 ml.

The effect of the concentration of stabilizer on the particle size was tested, and the result was as Figure 11. At the lowest stabilizer concentration, the average particle size was around 4 μm, which can be explained as the coverage of stabilizer on the microcrystal surface was inadequate to arrest particle growth. The particle size reduced with the increasing of stabilizer concentration. The particle size being inversely proportional to solubility, results indicated an increase in solubility at 0.05 % w/v stabilizer concentration.

Effect of Drug Content

ANOVA for Response Surface Quartic Model (Alliased)

ANOVA results for Response Surface Quartic Model (Alliased)

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	146.02	8	18.25	11.93	0.01498 significant
A-Drug Conc.	0.026	1	0.026	0.017	0.9017
B- Stabilizer Conc.	3.86	1	3.86	2.53	0.1872
AB	40.13	1	40.13	26.24	0.0069

A^2	35.9	1	35.9	23.47	0.0084
B^2	2.063E-003	1	2.063E-003	1.349E-003	0.9724
A^2B	20.10	1	20.10	13.14	0.0223
AB^2	0.18	1	0.18	0.12	0.7479
A^2B^2	57.46	1	57.46	37.57	0.0036

The Model F-value of 11.93 implies the model is significant. There is only a 1.49% 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 AB, A^2, A^2B, A^2B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

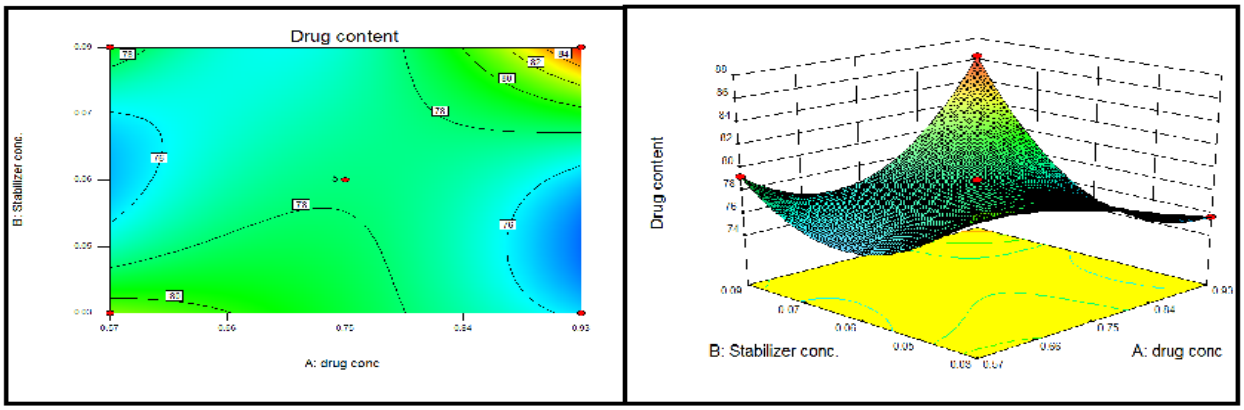

Figure 12 Effect of Stabilizer conc. and drug conc. on drug content

Final Equation in Terms of Coded Factors:

Drug content = +77.77 +0.081 * A -0.98 * B +3.17 * A * B -2.51 *A^2 -0.019 * B^2 +3.17 * A^2 * B +0.30 * A * B^2 +5.36 * A^2 * B^2

The effect of drug concentration on drug content was found to be positive. Thus with increase in concentration there was found to be increase in drug content. The most probable reason may be high drug loading leads to higher supersaturation and thus higher drug content.(figure 12)

The effect of stabilizer conecntration on drug content was found to be negative. Thus with increase in concentration there was found to be decrease in drug content. The most probable reason may be drug to stabilizer ratio which was decreased. (figure 12)

Effect on Drug Release

ANOVA for Response Surface Quadratic Model

Table 20 ANOVA results for Response Surface Quadratic Model

Source	Sum of Square	Degree of freedom	Mean Sum of Square	F value	p- value Prob> F
Model	115.21	5	23.04	4.13	0.0457 significant
A-Drug Conc.	9.94	1	9.94	1.78	0.2239

B- Stabilizer Conc.	2.74	1	2.74	0.49	0.5063
AB	50.34	1	50.34	9.02	0.0199
A^2	0.28	1	0.28	0.050	0.8300
B^2	52.02	1	52.02	9.32	0.0185
Lack of fit	26.85	3	8.95	2.93	0.1633 Not significant

The Model F-value of 4.13 implies the model is significant. There is only a 4.57% 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 AB, B^2 are significant model terms.Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 2.93 implies the Lack of Fit is not significant relative to the pure error. There is a 16.33% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

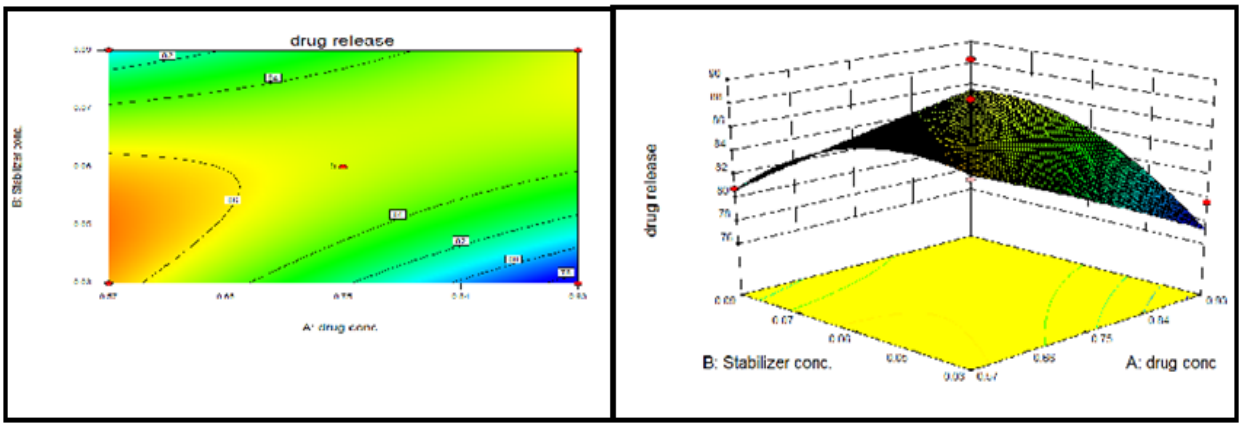

Figure 13. Effect of Stabilizer conc. and drug conc. on drug release

Final Equation in Terms of Coded Factors:

Drug release = +85.46 -1.11 * A +0.59 * B +3.55 * A * B -0.20 * A^2 -2.73 * B^2

Final Equation in Terms of Actual Factors:

Drug release = +104.97387 -39.29282 * drug conc -101.24492 * Stabilizer conc.+709.50000 * drug conc * Stabilizer conc. -6.38800 * drug conc ^{2} -3418.28125 * Stabilizer conc.^{2}

It can be seen that with an increase in drug loading, the particle size increased (Figure 13). High drug loading can generate higher supersaturation which favors more formation of nuclei, but which may cause the collision, agglomeration and growth of numerous nuclei within certain region. The particle size has direct impact on solubility according to Noyes Whitney equation. Thus the solubility was found to be increased with decrease in particle size which in turn caused improvement in dissolution profile.

The effect of the concentration of stabilizer on the particle size was tested, and the result was as Figure 13. At the lowest stabilizer concentration, the average particle size was around 4 μm,

which can be explained as the coverage of stabilizer on the microcrystal surface was inadequate to arrest particle growth. The particle size reduced with the increasing of stabilizer concentration. The particle size being inversely proportional to solubility, results indicated an increase in solubility at 0.05 % w/v stabilizer concentration. The drug release profile was improved with improved solubility.

CONCLUSION

Micron-sized drug powders can be prepared using a controlled crystallization technique in the presence of HPMC as protective hydrophilic polymer followed by spray drying. Solvent change method, using ultrahomogenizer and stabilizing agents produced microcrystals, with higher dissolution rate, compared to untreated sample. Changing the concentration of drug and stabilizing agent changed the size of crystals.

By the method used in this study, small drug particles are prepared without size-reduction techniques. Micronized poorly water-soluble drugs can be prepared by controlled crystallization of primary molecularly dispersed substances. The precipitation technique in the presence of protective hydrophilic polymers followed by spray-drying results in a drug powder with a markedly enhanced drug dissolution rate. A high and hydrophilized surface area is created. To form a protective layer on the crystal surface, the polymer must have an affinity to the hydrophobic crystal surface.

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